

Costs and Benefits of Clean Air

THE Organisation for Economic Cooperation and Development specializes in producing reports, but the organization's latest document, which sets out to assess the effects of an increased demand for energy on the environment, is out of the ordinary in that it is both lucid and a mine of useful information on sources of energy (*Report and Conclusions of the Joint ad hoc Group on Air Pollution from Fuel Combustion in Stationary Sources*, OECD, Paris, 1973). This, however, makes the report no different from several others which have appeared recently in what might be called energy concern year. But the intriguing aspect of the OECD document is a calculation of the likely costs needed to keep the level of gaseous pollutants from these sources at the same level in 1980 as it was in 1968, the base year for the OECD calculations. Between 1968 and 1980, according to all estimates, there will be a two thirds increase in total fuel consumption in most of Europe and in North America, while Japan during this period will increase its consumption by almost 230%. And if no action is taken to abate the gaseous pollutants from power stations and other industrial sources the level of these pollutants will increase by 70% by the end of the decade.

During the next ten years the price of all fuels, except possibly uranium and plutonium for nuclear power generation, will inexorably rise. And equally inexorably there are going to be pressures applied, quite properly, to keep the exhaust fumes from power stations and industry as clean and free of sulphur dioxide and nitrogen oxides as possible. But what increase in fuel costs should be tolerated in order to maintain the environment in its present state? There is no point in pretending that the consumer will not pay the increase in the end, for in spite of the often enunciated principle that the polluter must pay, the cost will appear on the electricity or gas bill, on the price of the product, or in some cases as an increase in taxes. But the news from the OECD is cheerful enough this time. Only an estimated 2.5% to 5% increase in fuel costs is necessary to keep sulphur dioxide emissions constant for the next few years. Such an increase is, of course, much less than the expected increase in fuel prices in the same period, and for once, the benefits of this increase in cost would seem to clearly outweigh the disadvantages.

But sulphur dioxide emissions do not constitute the entire story. Nitrous oxides and dust are also emitted from industrial stacks and the report does not consider the pollutants emitted from internal combustion engines. Unfortunately, the way in which nitrous oxides, which are one of the chief pollutants in car exhausts, contribute to chemical reactions in the atmosphere is not completely understood. And because of this the best way to treat these gases before they are emitted to the atmosphere is a contentious topic. But the technology is now available, at a cost, to reduce the amount of these oxides emitted even if little is known about how the oxides

cause problems. Although the cost of keeping sulphur dioxide emissions at a low level is modest when expressed in terms of the total spent on fuels it still means a capital investment of anything up to \$15,600 million, a not insubstantial sum, in the next few years and the annual extra operating costs would run to between \$2,200 million and \$4,200 million. But the OECD report raises the question of whether individual countries should decide to try and return the pollutant levels to those existing in 1968, or whether this should be an international approach. The OECD points out that it is feasible to control the amounts of sulphur dioxide emitted but the wider aspect is whether it is sensible to attack one part of the problem of air pollution while ignoring other substances which are being continually emitted into the environment. Certainly sulphur dioxide emissions should be contained within reasonable limits, but it is surely not fair that industries which burn fuels with a high sulphur content should be made to wear environmental halos while other industries which pollute in a different way should have no strictures put upon them. What is surely needed is an overall policy, similar to that for which the European Economic Commission has pleaded, which does not favour one side of industry over another.

100 Years Ago



Telescope Tube for Celestial Photography

I HAVE not yet seen any satisfactory plan suggested of getting over the difficulty experienced in celestial photography by the expansion and contraction of telescope tubes, by changes of temperature in metal tubes.

I therefore venture to suggest the following plan, which may be so arranged as to keep the object-glass and camera-slide exactly the same distance apart, and so keep the true focus when once found. The arrangement would have to be modified according to the metal of which the tube is made, but taking a brass one (the most common), with the camera attached to the eyepiece-slide, the correction will be effected by attaching to the main tube, near the eyepiece, two zinc rods the length of the main tube, upon which they must rest loosely; to the free ends of these, near the object-glass, attach a rod of iron extending to the eye-tube; let this iron rod be attached to the eye-tube when the sensitive-plate is exactly in focus; any change in temperature will then have no effect on the focus, for the expansion and contraction of the three metals will keep the distance from object-glass to sensitive-plate constant. All who have worked with a telescope giving sharp definition, will know that this is not an unnecessary precaution, as it may seem to some.

Sydney Observatory, June 14

H. C. RUSSELL

From *Nature* 8, 284, August 7, 1873.



OLD WORLD

The Research System as Margaret Thatcher sees it

FURTHER support for the formation of a European Science Foundation came from Mrs Margaret Thatcher, Secretary of State for Education and Science, in a recent address to the Parliamentary and Scientific Committee.

Mrs Thatcher said that increasing collaboration between European scientists creates the need for a "forum in which projects and ideas can be explored and exchanged". It is therefore "highly significant and encouraging that discussions which have been going on for the past few months between the research councils and academies of western Europe with the active participation of the European Commission seem likely to lead to the formation of an independent European Science Foundation to provide just such a forum".

Mrs Thatcher's public approval of the proposed foundation follows on that of Sir Brian Flowers, chairman of the Science Research Council, who recently declared that the formation of the foundation is now "inevitable" (see *Nature*, 243, 254; 1973). Like Sir Brian, Mrs Thatcher went on to emphasize that the "big sciences" would not always be the principal beneficiaries of such a foundation. "I am not keen . . . on cooperation merely for its own sake. But I do feel that if scientists are given support and opportunities to meet together in their own fields, there will be no shortage of worthwhile suggestions of good scientific merit, even though maybe of modest cost, for joint activity," she said.

Mrs Thatcher also discussed the changes that have followed from last year's white paper on *A Framework for Government Research and Development*. The white paper recognized, Mrs Thatcher said, that "scientific activity is one and indivisible. Much basic research will involve a capability for applied research and *vice versa* . . . It would therefore be a simple-minded view to say that the intention of the white paper was to assign responsibility to customer departments for the support of applied scientific research, and to regard fundamental science as an area in which the concerns of the government played no part. The reality is much more complicated. Some broad divisions can be made for the purpose of funding, but to my mind a more important feature of the white paper is the closer association of executive

departments with the totality of the research council's work".

"The essence of the research council system," Mrs Thatcher went on, "is that scientific judgments should be made by scientists and defended against the challenge of fellow-scientists. This is unchanged by the fact that scientists drawn from government now play a part".

BRITISH ASSOCIATION

Brighter Outlook

THE British Association for the Advancement of Science is shortly to launch an appeal for £15,000. Several hundred companies and charitable trusts are to be approached in the hope that they will agree to sponsor a wide range of the association's activities.

The proposed appeal is announced in the association's annual report published last week, and this week Mr Alec Hughes, an assistant secretary with the association, said that the intention is to invite companies to attach their names to particular activities and events in the association's calendar. Items for which the association is seeking support are the British Association Student Scientists conference planned for London in January next year, a full range of the British Association for Young Scientists activities, and the study groups that the association is setting up to examine "topics of urgent public and scientific moment".

The other prong of the BA's campaign is to approach universities, through their vice-chancellors, to ask their support. What the BA is looking for is both facilities for the association's branches to hold meetings, and some sort of active help from the universities in encouraging academics to join the association. One form this might take is the appointment of an official association representative on each campus. As Mr Hughes says, "the association's objectives in the advancement of science should be significant to the universities".

Although the target of the appeal is £15,000, only about a third of this is expected to be raised in the coming year. It is hoped that by inviting companies to sponsor particular events the response may be better than that which might meet a general appeal for money for unspecified purposes.

Discussing the role polytechnics are to play in research, Mrs Thatcher said their research "will be different both in nature and degree". Polytechnics may undertake some fundamental research, Mrs Thatcher said, as they may have particular expertise in certain subjects, but they "are and will remain" primarily teaching institutions. "While it would be right that their staff should engage in

In spite of the decision to launch an appeal the association's finances continue their recovery after the deficits of recent years. This year the accounts show a surplus of £2,242 on an expenditure of £48,792. The improvement is still largely due to administrative economies. Headquarters staff has been reduced to fewer than ten full-time administrators as against 24 two years ago. The move to more compact premises in Savile Row has also helped.

Sir Eric Mensforth, the association's treasurer, predicts that income will rise to £54,000 in 1973-74 "which should permit some welcome expansion in activities".

Membership expanded by 200 during the year to 1,900 members plus some 6,000 BAYS members, while plans to involve more students in the BA went ahead. This year's annual meeting is due to be held in Canterbury from August 20 to 24. The programme—on the face of things rather more stimulating than last year's—includes symposia on the exploration of the sea, on reserves, utilization and re-cycling of mineral wealth and one on Kent and Europe. Whales, oil pollution of the sea, earth resources satellites, the social responsibility of scientists and the future of weather forecasting will all be discussed, while Professor Kenneth Mellanby promises to demonstrate how almost all changes made by the government to improve the efficiency of research between 1945 and 1973 have been counter productive. Sir Kingsley Dunham's presidential address is on the advancement of environmental science.

As an experiment to encourage other learned societies and their members to become involved in the association's work the Challenger Society, the Geological Society of London, the Geologists' Association and the Estuarine and Brackish-Water Science Association are officially participating in some of the sessions.

research to strengthen their teaching abilities, I envisage the main research effort as being in a field to concern industry, business and the public services, often in a regional or even a local context. The universities will thus continue to be the main repositories of pure research conducted with the cultural aim of the advancement of knowledge for itself."

RESEARCH COUNCILS

New Chairman for NERC

SIR PETER KENT is to succeed Professor F. H. Stewart as chairman of the Natural Environment Research Council from October 1. Sir Peter, 60, who was knighted this year, is a former exploration manager and chief geologist of British Petroleum. His appointment is for a period of four years.

Sir Peter is no stranger to science policy. From 1968 until it was disbanded he was a member of the Council for Scientific Policy and he is currently an independent member of the Advisory Board for the Research Councils.



Sir Peter Kent

He comes to the Natural Environment Research Council at a time when it is having to adjust to the large scale reorganization of its work that emerged from last July's white paper on government research and development. Sir Peter was a member of the working group that produced the Dainton report on the research councils—which recommended a rather more evolutionary change in the relationship between the research councils and government departments than the large scale transfers of funds that Rothschild proposed and the government, after amendment, accepted. This year £2.25 million of the council's £15.3 million budget (1971–72

prices) were transferred to the Department of the Environment and the Department of Trade and Industry. A further £3.25 million and £4.5 million will have to be found in 1974–75 and 1975–76.

Sir Peter's experience with BP— which he joined in 1936—makes him well aware of the basic problems in geology and oceanography, as well as giving him an insight into the workings of industry that few academics achieve.

Sir Peter is a graduate of the Universities of Nottingham and London, a fellow of the Royal Society and a member of its council since 1968. While with BP he was responsible for geological survey work in Iran, East Africa, Papua, Canada, Alaska and Britain including the North Sea.

RADIOACTIVE WASTE

Safe in the Sea

REASSURANCE that the dumping of radioactive waste at sea is safe is given in a report issued yesterday by the National Radiological Protection Board.

Dr G. A. M. Webb and Dr F. Morley have built a model to evaluate how radioactive waste disposed of in the deep ocean is dispersed with time. By making all kinds of unlikely assumptions about the worst possible conditions for disposal, they confidently conclude that up to several thousand and, for some types of activity, several million times the quantity of radioactivity now disposed of in this way could be deposited on the sea bed without the dose limits recommended by the International Commission on Radiological protection being violated. Not only are the amounts of activity now being disposed of by Britain and other countries in this way negligible by comparison with the maximum allowed amounts, but Dr Webb and Dr Morley also point out that radioactive waste could be disposed of in the sea for several thousand years without there being any adverse effects.

A basic assumption made in the model is that the waste is dropped into deep water in packaged drums and that the drums reach the sea bed without any of the activity leaking out. In practice these drums are not airtight to ensure that during descent the pressure inside the drum remains the same as that outside so that there is no danger of the drum fracturing before it reaches the sea bottom. The different types of radioactivity are considered separately. The Harwell scientists assume that the tritium in the waste is released immediately the drum reaches the ocean floor whereas in practice it will probably take the tritium several years to percolate out of the drum. It is also assumed that all other waste will gradually leak out

within ten years although it is suspected that drums will retain their soluble waste for up to tens or even hundreds of years and indeed much of the waste could languish in the drums for much longer than that.

But the most significant assumption made is that the activity once released will not deposit onto sediments on the sea floor and that it will diffuse throughout the ocean. In practice most of the activity would be removed in this way and little would be left to be circulated.

Britain at present disposes of at most 20,000 curies of radioactive waste in the sea bed every year, of which only about 100 curies are due to alpha activity. According to the model it would be perfectly safe to deposit up to 10^{10} curies of alpha particle waste based on ^{239}Pu every year and up to 10^6 curies based on ^{226}Ra . Up to 10^{11} curies of general beta and gamma active waste could also be dumped with 10^{15} curies of tritium as well.

SPACE

Mars Soft-lander?

from our Soviet Correspondent

THE launch of Mars-4 and Mars-5, the latest Soviet Mars probes, on July 21 and 25, was announced with the usual reticence about their programmes. The official TASS press releases stated simply that their main aim was to continue "the scientific investigations of the planet Mars and its ambient space begun by the Mars-2 and Mars-3 probes in 1971", two probes "analogous in structure and purpose" are being used so that "simultaneous scientific investigations" . . . may "permit more complete data to be obtained". This does not imply, however, that the two probes are entirely identical.

The Mars-2 and Mars-3 probes, coming as they did almost ten years after the Mars-1 flyby, carried out an interesting series of synchronized observations of surface and atmospheric features from different orbits (that of Mars-2 being near-circular and Mars-3 highly elliptical).

It is difficult at this stage to estimate how the new probes will "continue" the programme. So far, the Soviet space planners have avoided straight-forward repetition of a successful project—thus Lunokhod-2 differed from Lunokhod-1 in carrying magnetic experiments and in traversing a far wider survey area. It seems likely, however, that some soft-landing attempt may be made. Whether this would be a simple physico-chemical instrument package, or one of the more exotic automated exobiology experiments or the roving "Marsokhod" that has been mentioned in the Soviet press, will probably remain a mystery until the probes reach Mars in February, 1974.

NEW WORLD

Fusion Power Changes Gear

by our Washington Correspondent

THE past year has been a very good one for fusion research in the United States. So good, in fact, that officials in charge of the programme at the Atomic Energy Commission are predicting that a demonstration commercial fusion power reactor will be operating by the mid-1990s—a five year improvement on their previous predictions. The factors which have led to such a rosy outlook are a run of successes in the research programme which have led to a recent re-orientation in planning, and most important, the possibility of a huge increase in the AEC's budget for such research in the next few years.

That, in any case, was the line taken last week by a string of witnesses before the Joint Committee on Atomic Energy, which held its second set of hearings in two years on the AEC's fusion research programme. In the previous hearings, AEC witnesses were optimistic about the future for fusion power (see *Nature*, 234, 120; 1971); this time they were positively enthusiastic, and their enthusiasm has been translated into plans to burn a fusion fuel of deuterium and tritium much earlier than they were estimating just a year ago.

Dr Roy L. Hirsch, Director of the AEC's Division of Controlled Thermonuclear Research, told the Joint Committee last week of a meeting of leaders of the programme held in mid-July, at which the outlines of the accelerated programme were agreed to. The chief change from previous plans is to push ahead with the design of equipment and experiments to burn a fusion fuel without first waiting for a demonstration that the physical conditions necessary to achieve a fusion reaction are scientifically feasible.

The ultimate goal of fusion research is to produce energy by imitating the processes that take place on the surface of the Sun. The idea is to heat a mixture of heavy isotopes of hydrogen—initially deuterium and tritium—to about 100 million degrees, and to contain the hot plasma out of contact with solid surfaces long enough for hydrogen ions to fuse together to form helium nuclei. The reaction would release energy and neutrons.

The previous plans called for a four-stage research and development programme to reach that goal. Phase one—the present phase—is physical research on methods of heating plasmas and confining them. The next stage is to build

experiments to demonstrate that it is scientifically feasible to reach the physical conditions of temperature, density and confinement time necessary to get net energy out of fusion reactions. This stage would involve research on hydrogen, which would not actually undergo fusion. The third step is to build a test reactor in which deuterium and tritium would be allowed to react to produce net energy output. And the final stage is the construction of a prototype commercial reactor. The new plan is to combine the second and third stages, a move which Hirsch said last week "reflects our growing belief that our successes indicate that scientific feasibility is not the milestone technical issue it was once thought to be".

Among the successes to which Hirsch referred are considerable advances in the past two years in the field of magnetically confining plasmas, but perhaps the most exciting breakthrough came early in July at Princeton's Adiabatic Toroidal Compressor (ATC), a tokamak machine. Last year, a series of successful experiments on the ATC machine produced good results in heating a plasma by compression, and in addition the density was increased to the minimum thought to be necessary to operate a fusion reactor. More recently, however, the Princeton machine has

been used to demonstrate a second heating method known as neutral beam heating.

First developed with the linear mirror machines at the Livermore Laboratory, neutral beam heating consists of injecting a high-energy beam of deuterium ions into the hot plasma. In the Princeton experiment, a plasma was heated to about 2 million degrees in the tokamak by resistance heating, compressed to raise the temperature to about 6 million degrees and then a beam of deuterium ions was fired into it. The trick is first to fire the deuterium ions through a gas so that they pick up electrons to neutralize the charge, which enables the beam to pass unhindered through the magnetic field that contains the plasma. The ATC experiments increased the ion temperature by between 20 and 25 per cent, even though the injected beam was at relatively low energy. "This early result", Dr Melvin Gottlieb, Director of the Princeton Plasma Physics Laboratory, said last week, "is indeed encouraging".

It is perhaps no coincidence that the reorientation of the fusion research programme was agreed to at a meeting which took place less than three weeks after President Nixon's statement on energy. In that statement, Mr Nixon announced that an extra \$100 million

NSF

David's Doubts

by our Washington Correspondent

DR EDWARD E. DAVID JUN., who resigned in January as Science Adviser to the President, expressed some reservations last week about the federal government's new science policy machinery which, he said, has "downgraded in my view, the direct influence of scientists on societal affairs". The new arrangements, announced two weeks after David left the government to become Executive Vice-President of Gould Inc., involve the abolition of the Office of Science and Technology and the transfer of its functions to the director of the National Science Foundation.

Testifying before the House Committee on Science and Astronautics, David suggested that the transfer of science policy responsibilities from the White House to the National Science Foundation has resulted in an "unstable" arrangement because "NSF's history is deeply rooted in the academic style which rightly demands single-minded

concentration on scientific excellence to the exclusion of other factors. This characteristic is difficult to leaven with other, less science-based realities".

He suggested that there are two possible resolutions of the instability, the first of which is for NSF to "follow its cultural past and thereby revert to the narrower concerns of science and academic research. . . . In this role, NSF would be no more than it is now". Alternatively, the foundation "may succeed in transcending its past, and actually achieve the national stature necessary" to carry out its coordinating and advisory functions. That, of course, would mean that the foundation would rise in the Executive hierarchy, since it would have to influence other government agencies concerning programmes which cut across their operating boundaries. "It would also mean", David suggested, "that NSF would serve as the technological beacon for other agencies. It must act as a surrogate for the President to exert these influences". David offered no predictions about which course the NSF will follow.

will be devoted to energy research and development next year, and that \$10,000 million will be spent on such research over the next five years. AEC Commissioner Dr Clarence Larson told the Joint Committee that the fusion programme would fit into the expanded energy research and development programmes, and Hirsch added that he expects the fusion effort to grow over the next few years into a programme costing "hundreds of millions of dollars". Last year, the fusion programme was given some \$66 million, this year, it is set to receive some \$90 million.

As for the balance of the programme, Hirsch said last week that tokamak research will continue to receive about 60 per cent of the funds, while the rest will be evenly divided between research involving mirror machines and the so-called theta pinch machines.

In view of the large expenditures envisaged for fusion research, and the optimistic forecasts for commercial fusion power, members of the Joint Committee predictably asked whether priorities in the AEC's budget need to be reassessed. In particular, should the commission continue to give the breeder reactor top priority? They received the predictable reply, for Dr Larson pointed out that it is reasonably certain that breeder technology will work, and that there will be full-scale production of power from such reactors by the year 2000. Fusion technology, on the other hand, although promising, is by no means certain, and that even if the goal of a demonstration reactor by 1995 is met, it will probably be another 15 years after that before widespread commercial power production.

In that regard, it is perhaps symbolic that while the Joint Committee was conducting its hearings into the fusion programme, Dr Dixie Lee Ray, the Chairman of the Atomic Energy Commission, was in Chicago for the signing of agreements between the government and private industry setting out the responsibilities of each party in the programme to build a liquid metal fast breeder demonstration reactor.

In his first energy message to Congress in 1971, President Nixon called the breeder the "best hope" for meeting energy demand, and the goal is to build the demonstration plant, at a cost of some \$700 million, by 1978. Following that, the AEC reckons that the reactor will be commercially available by the late 1980s.

It has often been argued, however, that by sinking so much money into the breeder reactor, other attractive methods of providing energy have been foreclosed, and in particular, the argument has been raised that fusion research has been held back through lack of money. A federal appeals court recently decided, however, that the AEC must file an

environmental impact statement on the entire fast breeder programme (see *Nature*, **243**, 431; 1973), which will require the commission to consider alternative methods of producing energy.

SCIENCE EXPENDITURE

Budget Battle Continued

by our Washington Correspondent

FOR the best part of a year, Congress and the Nixon Administration have been locked in a constitutional wrangle over which branch of the federal government has the final control over public expenditure. Although the issue has been rather overshadowed recently by a more pressing constitutional crisis, the importance of the budget dispute to the scientific community was made evident last week by a set of figures released by the House Commerce Committee. In the 1973 fiscal year—which ended on June 30—the Administration failed to spend nearly \$1,100 million that Congress had earmarked for health programmes, and the unspent money included some \$226 million for research programmes of the National Institutes of Health (NIH).

In fact, as far as NIH is concerned, the Administration totally ignored Congressional mandates and followed its own course of action. The situation is complicated by the fact that last year Congress did not pass an appropriations bill acceptable to President Nixon for the Department of Health, Education and Welfare, but, in short, this is what happened. After two HEW appropriations bills had been vetoed by Mr Nixon because he considered them inflationary, Congress finally decided that the department should be funded according to a continuing resolution. Such a resolution directs the Administration to fund any given HEW programme at the smallest level contained in one of three measures: HEW's budget for the previous year, the Administration's original budget request for 1973, or the vetoed bill. In the event, however, the Administration simply used a different measure—a revised budget request which, for the health programmes of HEW, happened to be \$1,100 million less than the continuing resolution stipulated.

Even the politically sensitive cancer and heart and lung programmes did not escape the knife—they were given \$59 million and \$44 million, respectively, less than the continuing resolution would have provided. And the rest of the institutes of NIH between them received some \$123 million less than the amount available.

Congress has thus been trying for several months to change the law to give itself a stronger grip on the purse-strings. Last week, the House of Representatives passed a bill which would

require the President to inform Congress if he intends to impound appropriated funds, and to release the funds if either the House of Representatives or the Senate directs him to do so within 60 days. The Senate had previously passed a stronger measure which would require that the funds be released as a matter of course unless both houses of Congress approve the refusal to spend the money. The differences between the two bills must be resolved by a conference committee, but President Nixon has already promised to veto whichever version finally emerges.

EXECUTIVE SHUFFLE

Train Moves On

by our Washington Correspondent

SINCE the Watergate scandal began to engulf some top officials in the Administration earlier this year, there has been much shuffling of Mr Nixon's team. Last week, it was the turn of Mr Russell E. Train, who was moved from the chairmanship of the Council on Environmental Quality to become Administrator of the Environmental Protection Agency. Train, who is completely untainted by the Watergate cesspool, replaces William D. Ruckelshaus, who was moved in April to head the FBI for a short time following the resignation of L. Patrick Gray. Ruckelshaus was also given a new job last week—he is now Deputy Attorney General.

Train's appointment to one of the hottest seats in the federal government has been greeted with some muted criticism from both sides—from environmentalists who fear that he will not be tough enough and from automobile manufacturers who fear the reverse. For his own part, Train said last week that he is his "own man", and that he will pursue a "strong, vigorous, fair enforcement policy".

The criticism that greeted his appointment is a fair taste of things to come, for Train will head the EPA during a critical phase of its existence. In virtually every area there are tough decisions to be made which Train acknowledged last week, "will test the commitment of the American public" to cleaning up the environment. One area in which particularly painful choices must be made is air pollution—the EPA has recently been issuing plans to meet the standards prescribed by the Clean Air Act which call for stringent cutbacks in traffic in some urban areas and for expensive emissions control devices to be fitted to automobiles. Mr Ruckelshaus used to say when he was Administrator of EPA that he was happy when his decisions were attacked from both sides and by that criterion, Train has started off on the right footing.

NEWS AND VIEWS

Leukaemia Viruses : Human or Feline ?

DURING a news conference at Los Angeles in 1971, Dr R. J. Huebner, of the US National Cancer Institute, announced the discovery of a C-type RNA virus in human tumour cells which, he said, represented a "light year" leap forward in the aetiology of human cancer. The virus which elicited the news conference was named RD-114, and details of its properties were subsequently published by McAllister *et al.* (*Nature new Biol.*, **235**, 3; 1972). RD-114 virus was isolated from a human rhabdomyosarcoma cell line which had been inoculated into the brain of a foetal cat, and from the beginning debate centred on whether RD-114 virus originated from the human sarcoma cells or from the foetal cat in which these cells were grown. The human origin was favoured because the internal proteins of the virus (group specific antigens and reverse transcriptase) were not immunologically related to those of previously described feline leukaemia viruses, and because the virus propagated could grow in human cells though not in feline cells. On the other hand, attempts to isolate similar viruses from subcultures of the same human sarcoma cell line which had not been passed through cats were unsuccessful, and no evidence of a viral genome related to RD-114 was found in these cells using molecular hybridization techniques. Neither did RD-114 virus seem to be related to the C-type viruses isolated from monkeys and apes.

Recently, the uncertain origin of RD-114 virus has been resolved on the side of the cat. In the May issue of *Virology* (**53**, 142; 1973), Livingston and Todaro describe the isolation from feline cells of a virus that is indistinguishable from RD-114 virus. The virus was spontaneously produced both by the CCC line of feline kidney fibroblasts and by other sublines of CCC cells which did not spontaneously release virus. Even normal feline embryo cells could be induced to produce the virus after treatment with 5-iododeoxyuridine. Thus RD-114 probably represents a latent virus of cats; when activated, it replicates more readily in human or primate cells than in CCC cells. In this respect RD-114 virus of cats resembles the endogenous viral genomes of chickens and mice. These viruses are known to be transmitted from one generation to the next as a set of "host genes", and they too are activated by 5-iododeoxyuridine to release infectious virus. Once activated, the chicken virus, like RD-114, replicates better in cells of other species.

Livingston and Todaro's discovery of an endogenous cat virus resembling RD-114 is supported by similar biological studies from Sarma's laboratory (*Nature new Biol.*, **244**, 56; 1973), and by a highly reiterated sequence of molecular hybridization studies (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1437; 1973, and *Nature new Biol.*, **244**, 51; 1973) from which Neiman's stands out for its clarity and lack of bombast. All these papers show that feline cells, but not human cells, contain nucleic acid sequences that are homologous to the RNA of RD-114 virus. The only remaining question on RD-114 is why several million dollars have been devoted to this particular virus. Each of these seven recent papers, independently demonstrating

the feline origin of RD-114, was supported by the US National Cancer Institute, all but one of them under contracts issued with the Special Virus Cancer Program (SVCP). But, it must be asked, whether the SVCP represents a better method of financing research than the traditional method based on peer review.

No doubt laboratories sustained by SVCP contracts will now turn to other "candidate" human tumour viruses and they might do worse than examine a modestly presented but convincing paper published last year (in English) by a Soviet group of scientists (Zhdanov *et al.*, *Arch. ges. Virusforsch.*, **39**, 309; 1972), which describes a C-type RNA virus isolated from a human leukaemia cell line. It is likely, however, that even when a virus is implicated in human leukaemia, it will be present in a latent form. A possible lead in the detection of latent virus is the report by Todaro and Gallo in last week's *Nature* (**244**, 206; 1973) that myeloblastic cells from a human patient with acute myeloblastic leukaemia have a reverse transcriptase possessing biochemical and antigenic properties closely similar to those of the enzyme of a C-type RNA virus isolated from an ape.

Whereas RD-114 virus is probably inherited, two reports by Jarrett *et al.* in the current issue of *J. natn. Canc. Inst.* and by Hardy *et al.* (*Nature*, **244**, 266; 1973) show that the ordinary feline leukaemia viruses (FeLV) are transmitted by "horizontal" infection. Indeed, even cats that are first exposed to infection as adults have a high chance of developing leukaemia and possibly other diseases. Thus leukaemia in cats is an infectious disease. Because FeLV infects human cells in culture, there is the possibility that the human population may be exposed to infection. This may also be true of RD-114 virus. A leukaemia virus that is transmitted genetically in one host strain or species may be transmitted by infection to another. The host carrying the integral viral genome could act as a reservoir hidden from the epidemiologist.

Hardy *et al.* point out that because FeLV is transmitted horizontally in cats, it may be possible to vaccinate cats against FeLV-associated diseases by inoculation with attenuated strains of virus. The potential of vaccination against infectious transmitted neoplastic disease has been proved with the successful vaccines developed against Marek's disease in domestic fowl, which is caused by a highly contagious herpes virus. It is generally assumed that no protection could be developed against leukaemia caused by "vertically" transmitted viruses because the virus is already established in the cells of the host. It is necessary to distinguish two fundamentally different modes of "vertical" transmission: inheritance of DNA proviruses integrated in the chromosomes of the gametes, and congenital transmission of infectious virus particles. In the latter case, one could envisage protection by maternal antibodies or by drugs which prevent the establishment of infection, for example inhibitors of reverse transcriptase. There is also evidence that immunological tolerance is not the inevitable consequence of genetic transmission.

From a Correspondent

PREDATION

Unpalatable Legumes

from our Plant Ecology Correspondent

MANY species of plant and animal have responded to intense predation pressure by developing toxic metabolites which render them unpalatable. The development in plant seeds of insecticidal compounds has highly selective value for ensuring survival and ultimate germination. A biochemical survey of various legume seeds, some of which are conspicuously free from insect predation, has now been carried out by Rehr, Bell, Janzen and Feeny (*Biochem. Systematics*, 1, 63; 1973).

In Central America and the southern United States, where the survey took place, legume seeds suffer attacks from a variety of Lepidoptera larvae and also from Coleoptera of the family Bruchidae. One abundant lepidopteran is the southern armyworm, *Prodenia eridania*, a polyphagous species which is known to be capable of consuming a wide variety of food plants, including many containing insecticidal toxins. These toxins are apparently detoxified by *Prodenia*. Its catholic appetite makes the southern armyworm an ideal test organism for use in bioassays of seed toxicity and palatability.

Rehr *et al.* ground a variety of legume seeds and offered them incorporated into an artificial diet to *Prodenia* larvae. Many of the seeds which did not suffer severe predation in the field contained high concentrations of unusual amino acids; these were extracted and then fed to the armyworms. This provided a means of identifying precisely the repellent compounds involved in seed protection.

Seeds of some legume species, for example *Cassia grandis*, which are heavily preyed upon in the wild, were found to contain no uncommon free amino acids. A diet containing ground *Cassia* seeds proved quite acceptable to the armyworms and produced negligible mortality. It is worth noting that this species produces large quantities of seeds, which presumably compensated for losses by predation.

Entada polystachya suffers moderate predation from insects in the field and the seeds of this species were found to contain an unidentified amino acid. A 5% ground diet of this species caused some pupal deformity in *Prodenia*, in which ventral abdominal segments were soft. Deformed pupae either died or produced adults incapable of copulation.

Legume species which were observed to be fairly free from predation, for example *Gymnocladus dioica* and *Albizia julibrissin*, contained high concentrations of unusual free amino acids, in these cases β -hydroxy- γ -methylgluta-

mic acid and albizziine respectively. A 5% *Gymnocladus* seed diet caused heavy mortality among the armyworms. They were strongly repelled by material containing 4% of β -hydroxy- γ -methylglutamic acid, which is the concentration found in *Gymnocladus* seeds. Normal L-glutamic acid at this concentration was neither repellent nor harmful to *Prodenia* larvae.

Evidently there is a highly selective advantage, in terms of seed survival, in the production and accumulation of certain amino acids. The protective system is most highly developed and most effective in plants such as *Gymnocladus* where toxin concentration is sufficiently high to be repellent to the predator even before consumption.

NEUROTOXINS

Receptor Function

from our Neurochemistry Correspondent

NATURALLY-OCCURRING toxins continue to be fashionable and powerful weapons in the armoury of molecular pharmacologists bent upon the analysis of receptors and factors influencing their formation and function.

α -Bungarotoxin has recently been used to "label" cholinergic receptors in cultured neurones from chick embryo sympathetic ganglia (Greene, Sytkowski, Vogel and Nirenberg, *Nature*, 243, 163; 1973). But the use of another toxin, histrionicotoxin, derived from a Colombian arrow-poison frog, has suggested that α -bungarotoxin may bind to a second site at the synapse in addition to the cholinergic receptor itself (Albuquerque, Barnard, Chiu, Lapa, Dolly, Jansson, Daly and Witkop, *Proc. natn. Acad. Sci. U.S.A.*, 70, 949; 1973).

The excitatory action of acetylcholine (ACh) at receptors on skeletal muscle involves two stages: first, combination with receptor sites, followed by an increase in the permeability of the post-synaptic membrane to sodium and potassium ions, resulting in a transient depolarization. As yet little is known about the increase in ion conductance, and how this is triggered by combination of ACh with its receptors. Albuquerque *et al.* now report, however, that the perhydro derivative of histrionicotoxin reversibly blocks ACh-induced potential changed at endplates in normal muscles as well as at extra-junctional sites in chronically denervated muscles, apparently by preventing the potassium conductance change, and, to a lesser extent, the sodium conductance change. Moreover, during successive ACh applications, the toxin becomes completely ineffective only after the first contact with ACh, suggesting that its action is dependent on an initial binding of ACh at the re-

ceptor. Thus, H_{12} -histrionicotoxin may be extremely valuable in the investigation of the "ionophore", although the ideal "label" would be an irreversible binding agent.

α -Bungarotoxin has previously been thought to affect neuromuscular transmission more or less irreversibly. But Albuquerque *et al.* report a partial recovery of the endplate potential after washout of α -bungarotoxin from poisoned diaphragm endplates. These results together with results from binding studies using a tritium-labelled derivative of α -bungarotoxin indicate two classes of binding sites, one occupied preferentially by curare, at which α -bungarotoxin binds irreversibly, and the other occupied preferentially by histrionicotoxin, at which the binding of α -bungarotoxin is reversible. Both sites are involved in synaptic excitation by ACh, and are equated by the authors with, respectively, the receptor site and the "ionophore".

More recently, Giacobini, Filogamo, Weber, Boquet and Changeux (*Proc. natn. Acad. Sci. U.S.A.*, 70, 1708; 1973) report how receptor blockade with a toxin from cobra venom affects the development of neuromuscular contacts in embryonic chick muscles. In their system toxin binding is confirmed to be specific for the ACh receptor site by its almost complete abolition by a variety of known cholinergic ligands. Chick embryos survive high doses of toxin and are therefore convenient for the study of the development of innervated muscle in the presence of toxin. In normal conditions the development of the motor innervation takes place in three main stages. First, from the fourth to the sixth day of incubation, motor nerve fibres invade the limb-bud muscles, and the first myotubes appear, with a diffuse localization of acetylcholinesterase. From the sixth to the twelfth day a massive formation of myotubes occurs, and acetylcholinesterase becomes restricted to the myotendon junctions. Finally, from the twelfth day onwards, muscle fibres replace the myotubes, and the nerve endings differentiate into motor endplates with a selective localization of acetylcholinesterase; at this stage action potentials can be seen, and the ACh sensitivity becomes restricted to the endplate region. Embryos injected with toxin were found to become atrophic with a marked reduction in the growth and differentiation of skeletal muscles—in 16-d embryos which had been treated with toxin on the 3rd, 8th and 12th day, the majority of contractile elements were still present as myotubes. Moreover, very few motor endplates could be seen, and the sciatic nerve, spinal roots and sensory ganglia appeared atrophic. It is unlikely that the toxin affects impulse propagation,

so these changes must be due to the lack of postsynaptic activation and hence of muscle contraction in the presence of toxin.

The fact that postsynaptic blockade causes degenerative changes presynaptically suggests that there is some transfer of information between the muscle cell and the nerve terminal during development. The authors rule out the possibility that this occurs via the neural feedback loop involving the sensory fibres from the muscle to the motoneurone by showing that in the extrinsic ocular muscles, which receive a purely motor innervation, toxin treatment still causes a reduction in the size of both the muscles themselves and the corresponding nerves.

The results suggest that information is passed directly from the postsynaptic to the presynaptic cell in the opposite direction to the transmission of impulses. Similar retrograde transsynaptic effects have been reported to take place in the brain after lesioning of particular areas (Cragg, *Brain Res.*, **23**, 1; 1970), and also in the cervical sympathetic ganglion, in which *in vivo* treatment with 6-OH-dopamine, which selectively destroys the postganglionic adrenergic neurones, results in a marked decrease not only of tyrosine hydroxylase, but also of the pre-ganglionically-located choline acetyltransferase (Black, Hendry and Iversen, *Brain Res.*, **34**, 229; 1971).

ECOLOGY

Cycling and Degradation

THE cycling of materials in ecosystems, with particular reference to chemicals and mineral wastes which may cause environmental deterioration, was the subject of the British Ecological Society's symposium held at Leeds University, July 10-12, 1973. Dr M. J. Chadwick (York University) discussed the significance of biogeochemical cycles in disturbed environments and illustrated the principles involved in the recycling of polluted river water through heathland and the nitrogen balance sheet of a colliery spoil. In the context of arable ecosystems, Dr R. G. Potts and G. P. Vickerman (Game Conservancy) suggested in their paper that although the quantity and diversity of pesticides used on farmland continue to increase, the ecological hazards were hard to identify and long term agricultural benefits were intangible. Causes of the reduction in the partridge population of 25 acres of downland were analysed and the biological significance of stability, diversity and predation in farmland ecosystems was discussed with reference to cereal aphids. Dr F. Moriarty (Monks Wood) reviewed the rate of release and subsequent dispersal

of DDT showing that the proportion of DDT found in living organisms is minute and that amounts probably depend on the physiology of each species rather than on its position in the food web.

In a paper by Dr A. Jernelov (WHO, Copenhagen) the global cycling of mercury using deposition data from the Greenland ice cap to monitor mercury contamination was critically examined. Although industrial emission into the atmosphere has increased the quantity of mercury in the environment, he suggested that changes in agricultural practices which stimulate microbiological activity in the soil increase the rate of methylation of mercury; and its subsequent volatilization could explain higher mercury contents in organisms without any increase in the total amount of mercury in the cycle. The nitrogen balance sheet in aquatic ecosystems was discussed by Professor W. D. P. Stewart (Dundee University). Using the scanning electron microscope to identify structural granules and polyphosphate bodies in algal cells, it was possible to assay the nitrogen and phosphorus status of the water. A substantial loss of nitrogen from the system was attributed to denitrification and low standing crops of algae to high levels of secondary production.

Contamination of the atmosphere with radioactive debris from nuclear explosions at various times and places and to various altitudes, and its redeposition, has been studied by Dr D. H. Peirson (AERE, Harwell) who in his paper explained on a meteorological basis the passage of material from the troposphere, or from the stratosphere through the troposphere, down to Earth. Dr A. D. Christie (Ontario University) followed with a paper on "Residence Times in the Atmosphere". Models used to interpret trace constituent distributions were critically evaluated.

Seventeen papers covered various aspects of air, water and land degradation. The use of biological surveys to detect pollutants by establishing biological patterns was critically examined by Professor R. W. Edwards and M. W. Read (UWIST, Cardiff) and in other papers methods of detecting pollution of air and water by non-ferrous metals were described. Interactions between pollutants complicated the interpretation of their possible biological effects and in discussion the need for thorough "ecotoxicological" studies was stressed to determine what levels of pollution mean in biological terms.

Factors inhibiting the recolonization by plants of colliery spoil, molybdenum tailings, uranium strip mined areas, pulverized fuel ash, kaolin clay spoil and chromate smelter waste were reviewed by American and British

workers. Nitrogen deficiency was a common feature in all these wastes but with adverse pH, specific ion toxicity and physical structure complicating revegetation procedure. Dr S. J. Wainwright (University of Wales) and Professor H. W. Woolhouse (Leeds University) explained how the complex mechanisms of metal tolerance in grasses involved a number of metabolic systems. Dr F. T. Carrucio (South Carolina) in a paper dealing with production of acidity in coal mine refuse showed how only one of four types of iron pyrite generated acidity and in conjunction with pH of ground water prior to mining activity could be used to predict potential acidity of spoil and plan remedial action during the mining operation.

The concluding session on "Planning for Resource Renewal" opened with a paper by Dr R. Curnow (Sussex University) which criticized many of the assumptions used in the MIT work on finite limits to growth. Papers by Dr G. Petsch (Ruhr Regional Planning Authority, Essen), C. V. R. Tandy (Land Use Consultants) and M. F. Downing and Professor B. Hackett (Newcastle-upon-Tyne) gave examples of rehabilitation of derelict landscapes and N. Jones and W. R. Howells (SW Wales River Authority) examined the role of natural agencies, remedial work and a hydro-electric scheme in improving water quality of a river affected by metalliferous wastes.

This symposium brought together a variety of experts involved in the scientific study of degraded environments. The published papers will provide specialist workers in this field and all who are interested in environmental quality with much factual material which would otherwise be less readily accessible.

POLYPEPTIDES

Aaargh!

from our Molecular Biology Correspondent
CALCULATING biopolymer conformations *ab initio* is not necessarily a reprehensible pastime so long as, to quote a legal view of a different vice, one does not do it in the street and frighten the horses. All the same, when the predictions are not readily testable they are scarcely relevant, if the answer is already known they are not very interesting, whereas if it is not known but experimentally accessible, the prudent man will generally prefer to wait and see, and perhaps not reflect overmuch on whether the theoreticians had spoken sooth, or merely registered a gallant near-miss. Whereas, from the very outset, calculations, even of a quite primitive nature, tended to yield satisfactory answers for the permissible combina-

tions of backbone torsional angles that are available to a polymer chain, that is to say in some wise supplanted model building as a means of restricting the stereochemical choice, the evaluation of the energies associated with different geometries remains, not surprisingly, an altogether more doubtful proposition. The class of homopolypeptides should offer by far the best hope of success, but the record in regard to experimentally defined systems is for the most part dispiriting. An interesting example relates to a new but hypothetical helix, first posited by Tiffany and Krimm on grounds of optical properties of charged polypeptides in water. This issue has stirred up the polypeptide field, not only because the discovery of a new, so to speak canonical, structure, where there was thought to be a random coil, would be a matter of some importance, but additionally because it bears on the thermodynamics and mechanism of protein denaturation and folding, and also on the appropriate standards to use for analysing the optical characteristics of proteins.

The circular dichroism of fully ionized polylysine and polyglutamic acid, which because of their solubility in water have been adopted as reference compounds for unstructured parts of proteins, shows a prominent positive feature at about 215 nm, which can be suppressed in high concentrations of some salts, giving place to what Tiffany and Krimm inferred to be the genuine random coil spectrum. The band at 215 nm they identified with a supposed extended state, which they equated with the left-handed 3_1 helix, characteristic of polyproline II. This contention they later supported with conformational calculations, based on the intramolecular coulombic interactions of the ionized groups. Subsequently, more elaborate calculations, taking into account the entire gamut of (in principle) calculable interactions, led another group of workers to reiterate the conclusion that the extended helix is indeed the preferred structure. It had meanwhile been pointed out that the circular dichroism of the charged polypeptides in water and dilute salt solutions could be perfectly simulated by introducing an admixture of α -helix into the random coil (the polymer being of course fully α -helical in the unionized state). These two opposing views have led to a veritable ping-pong match in the literature, and Rippon and Hiltner (*Macromolecules*, **6**, 282; 1973) have weighed in with some data on sequential polymers with glutamic acid in the repeating unit, together with alanine or alanine and glycine. These give qualitatively the same circular dichroism, with the same response to salt as charged monopolymers. Heating induces a shift in the same direction as salt. This is inter-

preted again in terms of an extended helix that melts to a true random coil. Calculations, the authors aver, also throw up the 3_1 helix as the most stable form.

Tiffany and Krimm (*Biopolymers*, **12**, 575; 1973) have now, however, started another hare: the denaturants urea and guanidine hydrochloride accentuate the positive band in the circular dichroism of the charged polymers, and generate a somewhat similar effect in denatured proteins at low temperature. They are undismayed by the consequences of their argument, namely that polypeptide chains in concentrated guanidine hydrochloride are not random coils but extended helices.

At this point one must surely expect the sleeping dragon to wake and roar, but the protagonists of extended helices have anyway now been badly mauled by Miller and his colleagues on both experimental and theoretical grounds. In the first place, Rao and Miller (*ibid.*, 835) state that commercial samples of polyglutamic acid and polylysine are commonly incompletely deprotected, that is to say the esterifying groups which are present on the side chains during synthesis have not all been re-

moved. When Rao and Miller prepare polypeptides with a residuum of 5% or so of aromatic ester groups, the positive Cotton effect is clearly in evidence. When the protecting groups are removed it vanishes completely. It is hard to imagine that this will be the end of the story, and a riposte will be awaited with interest. Rao and Miller then continue the demolition process by showing that whereas simple polyelectrolytes, as is well known, assume a stretched rigid conformation in the absence of supporting salts, the synthetic polypeptides, in which the charge sits at the end of a long side chain, undergo practically no such change, as judged by the relation between molecular weight and intrinsic viscosity. In a second paper, Rai and Miller (*ibid.*, 845) turn their attention to the calculations of conformation, and expose to view the frailty of the underlying assumptions. There are doubts, for example, about the effective dielectric constant to use in computing the charge interaction, in where to place the point charge, and in how to distribute the counterions. More, Rai and Miller note that the distribution of torsional angles along the backbone will depend

Revision of the Synaptic Circuit

For almost twenty years, the electrical model first described by Fatt and Katz (*J. Physiol.*, **115**, 320; 1951) has proved adequate to account for the action of a large number of substances on a great variety of chemosensitive membranes (Ginsborg, *Pharmacol. Rev.*, **19**, 289; 1967). In essence both excitation and inhibition are mediated by an increase in the conductance of a specific ion (or ions) through the neuronal membrane. (Both direction and magnitude of the ionic flow are dependent on a specific ionic gradient across the membrane and the change in membrane potential and resistance, so the model was readily testable as the use of intracellular micro-electrode became established.)

Perhaps Krnjevic and Schwartz (*Exp. Brain Res.*, **3**, 306; 1967) were the first to hint that synaptic transmission may involve other mechanisms when they reported that changes in membrane conductance were detectable during the depolarization of cortical neurones of the cat by acetylcholine (Krnjevic, Pumain and Renaud, *J. Physiol.*, **215**, 247; 1971). Later Weight and Votova (*Science*, **170**, 755; 1970) were able to show rather more conclusively that the slow EPSP of frog sympathetic ganglia is mediated by a decrease in membrane resistance. Unlike the classical EPSP described by the model, the slow EPSP was potentiated rather than decreased by the intracellular injection of depolarizing current. Further-

more it was decreased by hyperpolarization and finally reversed in direction at a level close to that of the equilibrium potential for potassium. They propose that the synaptically evoked excitation was the result of a decrease in the resting potassium conductance.

In next week's *Nature New Biology*, a paper by Paupardintritsch and Gerschlager is accompanied by two illustrations whose extremely elegant records show both excitatory and inhibitory potentials evoked by 5-hydroxytryptamine to be mediated by a decrease in membrane permeability. Again the reversal levels are such as to suggest that the excitation is mediated by a decrease in the resting membrane permeability to potassium and inhibition due to decrease in the resting influx of sodium. Less than a month ago Weight and Padjen (*Brain Res.*, **55**, 219; 1973) confirmed that the slow IPSP of the sympathetic ganglia also behaves as if it were mediated by a decrease in sodium permeability. They have likened this response to the hyperpolarization response of vertebrate photoreceptors to light (Baylor and Fourtes, *J. Physiol.*, **207**, 77; 1970; Tomica, *Q. Rev. Biophys.*, **3**, 179; 1970), and drawn attention to the results of Engberg and Marshall (*Acta Physiol. Scand.*, **83**, 142; 1971) and Siggins *et al.* (*Science*, **171**, 192; 1971) who attributed the inhibitory action of noradrenaline to an inactivation of the resting sodium conductance.

crucially on the degree of cooperativity of the conformational interconversion. There is in fact no reason to anticipate cooperative behaviour as regards the melting of the extended helix, and calculation of interactions over increasing distances along the chain indicates that there should also be no local order. Given that the conformational entropy does not actually favour an extended helical state over the coil, Rai and Miller conclude that all energetic factors that they can calculate should combine to produce a random coil: with the charges at the ends of the side chains, the interactions of neighbouring residues appear actually to have the opposite effect on the coil dimensions to that in polycarbonate for instance.

The circular dichroism is of course sensitive to conformation, and characteristic of the combination of torsional angles defining each well in the potential energy map. To the extent that the distribution of segments between these wells in the random coil will depend on the nature of the side chain, so also will the nature of the circular dichroism. At this stage one would guess that the 3_1 helix will ultimately go the way of the dreaded 3_{10} , that comet of ten or so years ago, which caused such turmoil in the field, only to vanish quite abruptly and with the merest whimper.

LIQUIDS

Molecular Motions

from a Correspondent

IN retrospect, the very successful meeting under this title organized by the Société de Chimie Physique at Orsay on July 2-6 could hardly have been better timed to take stock of this burgeoning field. About 160 workers attended and some sixty papers were heard, experimental topics being drawn from virtually the whole range of optical, acoustic, dielectric and particle-scattering methods now available for liquid studies. A limited number of purely theoretical papers were given, backed up by the latest in molecular dynamics calculations, the influence of which could be detected throughout most of the programme.

The meeting began in somewhat austere fashion, Professor J. Yvon (University of Paris) warning the participants with a certain *froideur* that classical liquids were non-existent, that they had better not forget that all the interactions they were concerned with were electromagnetic in character, that pressure is not quite the simple variable it seems to be and that, were it not for the Pauli exclusion principle, all liquids would be, at most, statistically neutral. The extent to which these admonitions were taken to heart was not immediately evident in what followed.

The focal point of the meeting was a set of "state of the art" lectures: P. Schofield (AERE, Harwell) on the theory of transport phenomena, J. Lamb (University of Glasgow) on ultrasonic relaxation, C. Brot (University of Nice) on correlation functions and orientational motion, S. Bratos (University of Paris) on vibrational motion in liquids, H. G. Hertz (University of Karlsruhe) on NMR applications, T. Springer (University of Jülich) on neutron spectroscopy and P. Lallemand (University of Paris) on light scattering.

In these lectures and the many related shorter communications certain themes recurred constantly and served to define the interpretive limitations which can still frustrate even the most spectacular advances in experimental technique. As expected, rotational motion dominated discussions, with emphasis on the newer results from infrared linewidths, depolarized Raman and Rayleigh-Brillouin scattering. One was conscious throughout of the still-expanding legacy of Gordon's 1968 paper on the extraction of rotational information from

infrared linewidth measurements which could be said to have inspired perhaps as many as a quarter of the papers presented.

As the contributions of Brot and Bratos succinctly demonstrated, the extraction of autocorrelation functions, now available independently for the first three spherical harmonics (dielectric, magnetic dipole or electric quadrupole and light scattering respectively), is reasonably well understood, though, even in favourable cases where a successful deconvolution of coupled processes is possible, the molecular information remains severely under-determined and many open questions remain. Brot cautioned against excessive reliance on either the Debye diffusion or the jump-diffusion models and urged that mechanistic conclusions be drawn with care, even when autocorrelation is clearly exponential.

It was perhaps the complete dominance of the language of autocorrelation and memory functions which distinguished this meeting from others with similar titles held several

OCEANOGRAPHY

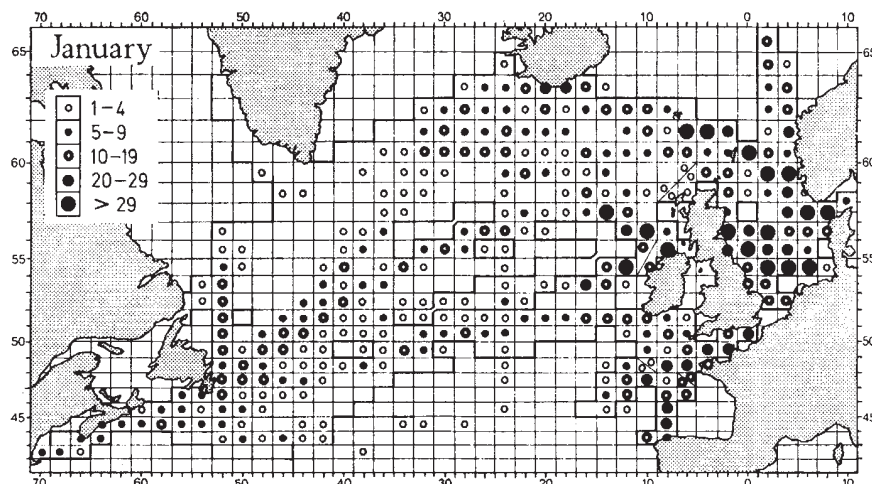
New Plankton Atlas

As a tribute to Sir Alister Hardy, who conceived and first developed the Continuous Plankton Recorder (CPR) in the early 1930s, the Edinburgh Oceanographic Laboratory (now part of the Institute for Marine Environmental Research and financed by the Natural Environment Research Council) has prepared a plankton atlas of the North Atlantic and North Sea (*Bull. mar. Ecol.*, 7, pp. xix+174; 1973) which provides charts of distribution of 255 species or groups of planktonic organisms averaged from monthly samples during the period 1958 to 1968. The recorders were towed at a depth of 10 m once in each month along several standard routes.

This map shows the number of

analysed samples in the standard rectangles of 1° latitude $\times$ 2° longitude taken in each January during the survey period. The numbers have been divided into five categories which are denoted by symbols (see key); the heavy lines delimit the sampled area. Many of the charts demonstrate the close association of species with the principal current systems of the North Atlantic.

At present, recorders are towed for more than 120,000 miles each year and by the end of 1971 ships of nine nations had towed the instruments for a total of 1,850,000 miles. The Natural Environment Research Council, with the help of the United States Government, is now financing the design and development of a new Undulating Oceanographic Recorder which will measure and record physical variables as well as sample the plankton in the upper 75 or 100 m of the oceans.



years ago. Although occasional dissenting voices were raised (Mansel Davies (University College of Wales, Aberystwyth) making an impassioned plea for explanations in terms of "individual molecules and what they are doing") it was clear that the majority of authors were quite at home with autocorrelation functions, even though the temptation to see in them the more picturesque mechanisms of earlier times was clearly irresistible to some. There was, in fact, a certain groundswell of reaction against the established physico-chemical vocabulary of "activation energies", "collisions" and "migrating defects" and a noticeable insistence on cooperative motions and coupling. Translation-rotation coupling was frequently alluded to, though there appeared to be a certain confusion between simple statistical correlation and actual mechanical coupling—the latter being presumably a truism, given conservation of angular momentum.

A regrettable consequence of the tight programme was that there was little opportunity to learn in detail about the newest experimental techniques. Remarkable among the short contributions were the photon-correlation light-scattering work of Demoulin, Ostrowsky and Montrose (ENS and Catholic University, Washington), the use of low-frequency depolarized light scattering to obtain collective orientational correlation times by Gershon and Oppenheim (University of Beer Sheva), the measurement of anisotropy in rotational motions by J. E. Griffith (Bell Labs), the ingenious combination of results from NMR and neutron spectroscopy by M. D. Zeidler (University of Karlsruhe), the pressure-dependence of Rayleigh scattering by Dill and Litovitz (Catholic University, Washington) and the use of paramagnetic ESR probes for the study of liquid motions by Martini, Romanelli and Burlamacchi (University of Florence). If tentative conclusions can be drawn, they might be that the newer optical techniques have established an edge over those of NMR, conventional ultrasonics, dielectric dispersion because of their more favourable place in the time-scale of molecular processes except where special, chemically interesting systems are concerned.

Nevertheless, optical methods did not have complete monopoly of experimental virtuosity. A. Mills (ANU, Canberra), for example, described how sense is at last being made of the conflicting data on self-diffusion coefficients, particularly for water. Results to date indicate almost beyond doubt that the square-root law of mass-dependence does not apply to isotopic variants HDO and HTO.

Dielectric measurements, though less conspicuous than in earlier times, continued to be described, though with a

certain shift of emphasis towards non-dipolar liquids. Mansel Davies discussed FIR absorptions in molecules such as CS_2 , CH_4 , benzene and so on, whereas dielectric absorption by n-alkanes in the range 26-140 GHz had been measured by U. Stumper (University of Göttingen). On the theoretical side R. H. Cole (Brown University) outlined a new derivation of Glarum's formula for the dipole correlation functions from microscopic arguments which again challenged the alternative result of Fatuzzo and Mason.

An afternoon was devoted to expositions of recent "molecular dynamics" calculations by two leading exponents, L. Verlet (Orsay) and A. Rahman (Argonne National Laboratory). Verlet's contribution concerned mainly simple liquids, for which a major result is the now indisputable long tail to the velocity autocorrelation function — believed, though still not demonstrated (except in two dimensions) to indicate hydrodynamic, vortex-like motions behind a moving atom. The implications of this for the computation of transport coefficients were taken up by Schofield,

while Brost offered the engaging possibility that a rotating molecule might "lose" memory of its immediate past, then regain it after a hydrodynamic delay. If the microscopic behaviour of liquids is really as perverse as this there will be much scope for future meetings.

A. Rahman described a recent simulation of 216 molecules of water using an improved version of the Ben-Naim-Stillinger potential. He and Stillinger interpret the results as offering little evidence either for phonon-like behaviour, or jump diffusion, or Gaussian self-correlation in the proton motion, and equally little support for collective behaviour in the form of clusters, non-bonded regions and the like.

Though perhaps, in the end, exposing more gaps than it was able to fill in the connexion between theory and experiment, the meeting demonstrated overall the very considerable progress which has been made, not only in understanding but in defining what one might reasonably expect to understand of the microscopic physics of non-simple liquids.

Conductivity Anomalies by Partial Melting?

FROM their measurements of geomagnetic variation fields, Reitzel *et al.* (*Geophys. J.*, **19**, 213; 1970) and Camfield *et al.* (*Geophys. J.*, **22**, 201; 1971) recently demonstrated that there are large electrical conductivity anomalies (enhanced conductivity) in the depth range 50-400 km beneath the western United States. Beneath the Basin and Range Province and under the Southern Rocky Mountains of Colorado, for example, conductivity in the upper mantle can vary by as much as a factor of 100 within conductive structures which include an elongated ridge on the surface of the conductive mantle and a combined step and ridge.

But what causes such large conductivity highs? In next Monday's *Nature Physical Science* Chan and colleagues consider a possible origin for the anomalies which is suggested by the correlations with other geophysical phenomena. Other workers have shown that the conductive structures beneath the western United States correlate strongly with, for example, heat flow and seismic wave velocity and absorption. But, as Hales and Doyle (*Geophys. J.*, **13**, 403; 1967) have pointed out, regional variations in P wave and S wave velocity in the upper mantle can be explained most reasonably by a small amount of partial melting in the low velocity zone. Is it possible, then, that partial melting can also account for the conductivity anomalies?

Chan and his colleagues thus examine this idea in the light of theories explain-

ing electric conduction in molten silicate materials. Specifically, they investigate the effect of 0.75-3.7% of partial melting on bulk conductivity in a situation where the melt has a much higher conductivity than the unmelted matrix. It turns out that if 1% melting is to increase the bulk conductivity by a factor of 100, the conductivity of the melt must be about 10^4 times higher than that of the matrix; and so the problem is to find a mechanism which in, for example, olivine will increase the conductivity by such a factor on melting.

After considering increases in ionic diffusion rates and intrinsic electronic conduction, Chan *et al.* come down strongly in favour of a mechanism involving hopping between localized states of Fe^{3+} and Fe^{2+} (polaron hopping) because the conductivity of olivine is known to increase rapidly with increasing $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio (oxidation). This is analogous to impurity conductivity in semiconductors at low doping concentrations. On the other hand, the conductivity of the liquid melt should also be higher (but not by a factor of 10^4) because of a higher iron concentration there than in the matrix and the higher diffusion rate of Fe^{2+} with respect to Mg^{2+} . Chan and his colleagues thus conclude that several mechanisms probably act together to account for the conductivity increase; but whatever these mechanisms are, a bulk conductivity increase by a factor of 10^2 because of partial melting seems quite feasible.

HIGH ENERGY PHYSICS

Anti-proton Production

from a Correspondent

THE possibility of matter and antimatter co-existing inside a galaxy is suggested in some cosmological models such as that of Alfrén (*Rev. mod. Phys.*, **37**, 652; 1965). The presence of a source region of antimatter somewhere in the Galaxy would be implied if an appreciable quantity of antimatter was to be detected in the primary cosmic ray flux. Experiments reported to date have only been able to yield upper limits to the proportion of antiparticles ($\lesssim 0.1\%$). Indeed no one seems to have positively identified even one antinucleon reaching the vicinity of the Earth.

Although 90% of cosmic ray particles are known to be protons many experiments concentrate on looking for antihelium and heavier antinuclei rather than antiprotons. This is to avoid the problem of having to correct for the secondary flux of antiprotons produced in the collisions of primary cosmic rays with interstellar gas. Experiments (for example looking for antihelium using superconducting magnetic spectrometers flown in balloons) therefore generally suffer from lack of a significant number of events (see, for example, Buffington *et al.*, *Nature*, **236**, 335; 1972).

A considerable improvement in statistics could be achieved by measuring the antiproton to proton ratio, provided the contamination due to secondary effects can be accurately calculated. Previous attempts at such calculations have suffered from the lack of sufficient information on the antiproton ($\bar{p}$) production cross-section ($p + \bar{p} \rightarrow p + \text{anything}$). Recently, however, T. K. Gaisser and R. H. Maurer (*Phys. Rev. Lett.*, **30**, 1264; 1973) have used data now available on $\bar{p}$ production from the CERN intersecting storage rings (ISR) to calculate the expected $\bar{p}/p$ ratio due to collision effects. Their results place a conservative upper limit of $\bar{p}/p \lesssim 4.6 \times 10^{-4}$ arising from interstellar gas collisions at all energies.

Drs Gaisser and Maurer have derived an expression for $\bar{p}$ production based on both conventional accelerator data and that from ISR. Interpolation between these sets of data and extrapolation beyond ISR energies (> 53 GeV in the centre of mass frame) are carried out on the basis of the scaling hypothesis of Feynmann.

The $\bar{p}$ production inclusive cross-section is then used to calculate the expected $\bar{p}/p$ ratio. Because the mean path length of matter traversed by primary protons between their source and observation at Earth ($\sim 3\text{--}5$ g cm $^{-2}$) is much less than the interaction length for protons in hydrogen, the authors assume that each proton interacts at the

most with one hydrogen nucleus, and also neglect further interactions of the produced antiprotons. With the further assumptions that the primary cosmic ray flux in interstellar space is primarily protons with the same energy spectrum as observed in the vicinity of the Earth and that $\bar{n}$ production equals $\bar{p}$ production, an expression for the differential spectrum for antiprotons is derived. Feeding in a power law primary cosmic ray energy spectrum ($dN \propto KE^{-2.6} dE$) yields the expected $\bar{p}/p$ as a function of antiproton energy.

Their calculations yield a $\bar{p}/p$ ratio initially increasing with energy but approaching an asymptotic limiting value of $\sim 4.6 \times 10^{-4}$ above ~ 100 GeV. Drs Gaisser and Maurer emphasize that the result is very much a firm upper limit to the $\bar{p}/p$ ratio at all energies. They argue that the final asymptotic figure is either insensitive to the approximations used or can only be reduced by more accurate treatment.

The authors' conclusions do, however, seem to depend on the correctness of the scaling hypothesis. Most recent ISR work and data from cosmic ray workers have raised considerable doubts concerning the applicability of scaling. Thus there is an obvious need now to improve the experimental results to below the current upper limit of $\sim 10^{-3}$ for the $\bar{p}/p$ ratio. Sorting out such a small proportion of antiprotons from protons, however, is a formidable task.

LOW TEMPERATURE PHYSICS

Another Sound

from our Condensed Matter Correspondent

A COMPLETELY new type of sound wave may exist in liquid helium-four above its superfluid transition temperature, T_λ , according to a calculation by D. C. Mattis and L. F. Landovitz of Yeshiva University, New York (*Phys. Rev. Lett.*, **30**, 1196; 1973).

The properties of liquid helium at temperatures above and below T_λ are so completely different that the two phases are distinguished by referring to them as He I and He II respectively. Most of the research effort has been directed towards reaching an understanding of He II whose superfluidity and other "anomalous" properties have made it a substance of intense fascination for both theoretical and experimental physicists. In comparison, He I has hitherto been regarded as relatively uninteresting, because its behaviour has seemed very similar to that of a dense classical gas.

It has long been known that in He II there are two entirely different modes of wave propagation, referred to as first and second sound, and travelling with very different velocities. First

sound is characterized by density fluctuations as in "ordinary" sound and by the superfluid and normal component of the liquid moving in phase with each other, whereas in second sound the two components move in antiphase, so that the net density remains constant. Because the proportion of superfluid falls to zero at T_λ , it has always been tacitly assumed that the only form of wave propagation in He II must be ordinary sound. Experiments have shown that, as expected, the velocity of sound in He I is not greatly different from that of first sound in He II, and that it can be measured continuously through the transition; but that the velocity of second sound falls rapidly to zero as the temperature rises to T_λ .

Mattis and Landovitz have now carried out a theoretical investigation of He I by considering collective excitations of the system, that is those involving the correlated motion of large numbers of atoms. Their calculation shows that, at temperatures above T_λ , there should be, in addition to ordinary sound, another much more slowly propagating wave.

For temperatures close to T_λ this wave should have the novel (for He I) character of being transverse, that is, the atomic motion being at right angles to the direction of propagation. With increasing temperature, however, the velocity of the wave rises and the character of the disturbance gradually becomes less well defined until, eventually, at a critical temperature T_b , the wave becomes indistinguishable from ordinary (longitudinal) sound. For temperatures above T_b the equations do not yield a wave solution. The authors suggest that T_b probably corresponds to the boiling point. In support of this thesis they report that the heat capacity, calculated on the basis of the same model, displays a "bump" at T_b ; and they believe that, if account could be taken of the weakly attractive interatomic forces, a latent heat would then be found, characteristic of the phase change from liquid to vapour.

Another interesting feature of their calculation is that, as the temperature falls, the velocity of the new wave drops to zero at T_λ . The fact that, in He II, the velocity of second sound also falls to zero but as the temperature rises to T_λ suggests that, in some sense, the new sound wave represents a continuation of the second sound mode of the He II, into He I.

The theoretical approach suggested by these authors may, perhaps, have laid the foundations for a new unified theory of both He I and He II. The most encouraging demonstration of the general validity of their ideas would be an experimental observation of the new wave-mode which they have predicted in this paper.

Ancient Linkage Groups and Frozen Accidents

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Evidence is presented that the genetic differences between man and the other mammals are extremely small. This supports the concept that natural selection conserves the *status quo*. The idea of evolution viewed as a succession of "frozen accidents" is discussed, taking as an example the remarkable similarity of the X-linkage group in all mammals both placental and marsupial.

No one would argue that the central concept of biology which congeals divergent disciplines into one cohesive whole is the Darwinian concept of evolution. Evolution, in turn, is contingent on natural selection. It is then of the utmost importance that we do not misunderstand the nature of natural selection. At first, natural selection was thought of as an omnipotent advocator of progressively adaptive changes. Those who still hold such a view see evolution as a relatively fast process, all inclusive in nature and affecting the entire genome. More recent amino acid sequence analyses of a large number of direct gene products, on the other hand, indicate that the true nature of natural selection is most often that of an avid conservative extolling the virtue of *status quo*. Could it be that extensive adaptive radiation of mammals actually involved only a very small fraction of the functional gene loci, while the rest remained relatively unmolested? If so, the genetic difference that separates man from other beasts might be surprisingly small.

Several years ago, my colleagues and I made two postulates on the genetic content of human chromosomes: (1) As an evolutionary freeze must have been placed on the X chromosome of a common mammalian ancestor, any gene locus which is found to be X linked in man must automatically be X linked in all other mammalian species¹⁻³. (2) At the stage of fish or amphibian, a mammalian ancestor must have undergone at least one round of tetraploid evolution. Consequently, a homologous relationship may still be found among pairs of apparently nonhomologous human chromosomes⁴⁻⁶.

Since then, cytological as well as genetic evidence in favour of those two postulates has steadily accumulated. Here I shall discuss the extremely conservative nature of natural selection and the concept of frozen accidents within the context of human evolution and human linkage groups.

Man and Apes

Differences between man and apes are obvious to the naked eye, yet such differences need not have involved a large number of gene loci in adaptive changes. Although man is said to be a naked ape⁷, a full set of fur can apparently be restored to the human body by a single dominant mutation⁸. At each polymorphic gene locus, natural selection can favour a certain genotype only by eliminating other alternative genotypes as unfit. Accordingly, a population which is undergoing rapid adaptive changes has to pay a considerable price for each gene locus which is subjected to selection. This is what Haldane apparently meant by the "cost of natural selection"⁹. It follows, then, that successful adaptive changes cannot involve too large a number of gene loci, otherwise a population would be exterminated by natural selection while undergoing these changes. Although polymorphism has been documented for about 33% of the human gene loci tested⁹, the very fact that human populations are not in the slightest danger of extinction suggests that natural selection has been oblivious to these polymorphisms; polymorphic alleles most often representing functionally neutral mutations¹⁰. It would not be surprising if most of the gene loci in the genome were not involved in adaptive radiation which separated man from apes. Indeed, haemoglobin α as well as β chain amino acid sequence is identical between man and the chimpanzee, while the gorilla differs by one amino acid substitution at each of these two chains¹¹.

Evolutionary Rates

Inasmuch as a greater number of the sequenced peptide chains have undergone evolutionary changes at rates slower than that for haemoglobin chains, one would expect that at many gene loci there would be no detectable difference between man and apes. Although *samesense* mutations cannot easily be detected by sequence analysis of peptide chains, the expected ratio between *missense* and *samesense* mutations is only 17:6. Expressing the rate of evolutionary changes in terms of accepted point mutations (PAM)/100 amino acid residues/100 million years (m.y.) (which is about the time for which mammals have existed on Earth) the value obtained for haemoglobin chains is 14, whereas lower values of 0.06 for histone IV, 3 for cytochrome c, 4 for insulin and 5 for trypsinogen have been obtained¹¹. Evolutionary conservatism is indeed evident at many of the gene loci.

One might argue that natural selection in the past has elevated most of the gene loci of higher vertebrates to states of near functional perfection, and that this is the very reason we no longer see much evidence of adaptive changes in many gene loci of man and apes. Indeed, so far as haemoglobin

chains are concerned, the rate of evolutionary changes appears to have slowed down noticeably in higher primates¹². Yet such an argument is revealing only a part of the truth and merely restating the point which I made earlier that natural selection can play the role of advocator of adaptive changes only on a very small fraction of the functional gene loci in the genome. One can easily argue that *Escherichia coli* or any other contemporary organism in its own way represents a state of near functional perfection.

The rate at which an organism evolves is greatly influenced by its generation time; the longer the generation time, the slower the rate of evolution. Man, with a generation time of 15 yr, has gone through only 330 generations in 5,000 yr, whereas the mouse with a generation of 2 months would have gone through 30,000 generations in a comparable time. The observed evolutionary slowdown in higher primates may simply be the consequence of progressive prolongation of their generation time.

The X-Linkage Group

"Frozen accident" is the term coined by Crick to explain the universality of codons¹³. Codons on messenger RNAs owe their very existence to anticodons on transfer RNAs. The triplet coding system as we know it need not represent an ideal solution for the translation of a nucleic acid base sequence to an amino acid sequence of a peptide chain. Nevertheless, at the very beginning of life on Earth, there occurred a functional coupling between messenger RNAs and transfer RNAs based on a triplet codon-anticodon recognition. Once this occurred, an immediate evolutionary freeze had to be placed on the system, for any subsequent change in the coding system would necessarily have made a mockery of all previous messages, thus resulting in extermination of any living form which dared to attempt a change.

In fact, evolution can easily be viewed as successions of frozen accidents. An enzyme seldom functions alone. Most often a number of enzymes are functionally coupled together to constitute one metabolic system. Once a reasonably well-functioning system was established, any drastic change in the kinetic property of one enzyme, without concomitant adjustments in the kinetic properties of functionally coupled enzymes, would tend to be disastrous. Mutations being rare and random events, the chance of two or more independent gene loci concomitantly sustaining mutually acceptable mutations is practically zero. Hence, the reason for an evolutionary freeze of the entire system.

This concept of frozen accident applies as well to the observed conservation by diverse mammalian species of the X linkage group *in toto*. The unique dosage compensation mechanism for X-linked genes which relies on the functional inactivation of one or the other Xs in female somatic cells¹⁴ seems to have evolved in an ancestor common to both placental and marsupial mammals. Thus the mechanism must be nearly 100 m.y. old.

The invention of this particular form of dosage compensation mechanism would have necessitated the conservation *in toto* of the original X chromosome in all the descendants of that common ancestor, for the only acceptable X-autosome translocation would be a terminal fusion between the whole X and an autosome which brings about the so-called XY₁Y₂/XX scheme of sex chromosome constitution. All other forms of X-autosome translocations which fragment the original X to separate pieces would disturb the established dosage compensation mechanism, and, therefore, be eliminated by natural selection. Indeed, the X chromosome of most mammalian species seemed to be of the same absolute size comprising

about 5% of the genome. Exceptionally large X chromosomes seen in certain species such as the golden hamster, *Mesocricetus auratus*, and the field vole, *Microtus agrestis*, were found to carry excessive baggage in the form of genetically empty, constitutive heterochromatin¹.

X Linking in Other Mammalian Species

Is any gene locus which is found to be X linked in man automatically X linked in all other mammalian species? The list of gene loci which were found to be X linked in more than one mammalian species was originally very slim^{2,3}. With the increased use of the electrophoretic technique as well as interspecific somatic cell hybrids for linkage analysis, however, the list has rapidly grown to a more convincing proportion, as shown in Table 1. One may now accept the postulate as the fact that the X chromosome of any mammalian species, regardless of whether it be placental or marsupial, is the exact or near exact genetic equivalent of the human X chromosome. The X-linked genes listed in Table 1 have no direct connexion whatsoever with the act of sex determination. It is merely an evolutionary accident that a chromosome which happened to carry those genes was selected to become the X chromosome in an ancestor of mammals and that the subsequent invention of the particular form of dosage compensation

Table 1 X Linkage in More Than One Mammalian Species

Human X-linked genes	X linked in other mammals	References
Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49)	Horse, donkey Hare Mouse Kangaroo	18, 30, 31 32 33 34
Phosphoglycerate kinase (E.C. 2.7.2.3)	Horse Kangaroo	18 35
Hypoxanthine-guanine phosphoribosyltransferase (E.C. 2.4.2.8)	Horse Mouse	18 33
Phosphorylase kinase (E.C. 2.7.1.3)	Mouse	36
Antihæmophilic factor viii	Dog	37
Antihæmophilic factor ix	Dog	38
X _G erythrocyte antigen	Gibbon	39
Dominant hypophosphataemic rickets	Mouse	*
Anhidrotic ectodermal dysplasia	Cattle	40

Those which are found to be X linked in two or more non-human mammals are not included. In order to avoid a larger reference list, those referring to X linkage of the above genes in man are omitted.

* In the linkage map of the mouse. Distributed by M. C. Green, Jackson Laboratory, Bar Harbor, Maine (1972).

mechanism put an evolutionary freeze on the entire linkage group. There seems to be no better example of a frozen accident in evolution than the mammalian X-linkage group.

Various new staining techniques permit the visualization of characteristic banding patterns on individual chromosomes. It has been shown that when stained with quinacrine mustard¹⁵, the human X chromosome shows three brightly fluorescent spots; one in the middle of the short arm and two on the long arm¹⁶. Although the observation that the same banding pattern is seen in various primate X chromosomes is, by itself, not too surprising¹⁷, it is of more interest to note that the same banding pattern is also observed on the horse X chromosome¹⁸ (Fig. 1a). It may be that even the original linear order of these X-linked genes was conserved to a remarkable degree during mammalian evolution, although the very fact that some X chromosomes, such as that of the mouse, are acrocentrics reveals that pericentric inversions did occasionally occur.

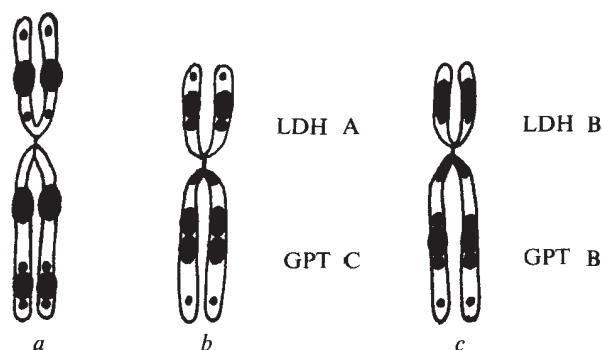


Fig. 1 *a*, Stylized quinacrine mustard banding pattern characteristic of the X chromosome of not only man and higher primates but also of the horse^{17,18}. Also shown is the stylized quinacrine mustard banding pattern of human chromosomes 11 (*b*) and 12 (*c*). In spite of a difference in the centromere location, the overall similarity in their banding patterns is quite striking.

Homologous Relationship between Non-homologous Autosomes

So long as a particular function is assigned to a single gene locus in the genome, natural selection jealously conserves the active site of that gene product. Thus, a trypsinogen gene would forever remain a trypsinogen gene, and a cytochrome *c* gene would remain a cytochrome *c* gene. How, then, were successive additions to the genome of new gene loci with previously nonexistent functions made possible during vertebrate evolution? The mechanism of gene duplication provides a temporary escape from the relentless pressure of natural selection to a duplicated copy of a functional gene locus. While being ignored by natural selection, a duplicated and thus redundant copy is free to accumulate all manner of randomly sustained mutations. As a result, it may become a degenerate, nonsense DNA base sequence. Occasionally, however, it may acquire a new active site sequence, therefore a new function and emerge triumphant as a new gene locus. This new locus would again be placed under the protective shelter provided by natural selection, so that there would be no further accumulation of mutations which would surely lead to degeneracy.

Gene duplication can be accomplished either by piecemeal tandem duplications involving one small chromosomal segment at a time, or in one fell swoop by tetraploidy. Tandemly duplicated genes should form a closely linked cluster. Indeed, in mammals, such a cluster is found with regard to haemoglobin β , γ , δ -chain genes as well as in genes for various classes of immunoglobulin heavy chains; γ , α , μ and so on. Inasmuch as functional gene loci would comprise only a very small fraction of the genomic DNA¹⁹⁻²¹, however, tandem duplications would more often result in the accumulation in the genome of nonfunctional DNA rather than in duplication of functional genes.

Tetraploidy

Tetraploidy, on the other hand, duplicates every functional gene locus simultaneously, and the genes duplicated by tetraploidy should initially be unlinked from each other, although loose linkage may subsequently be established by chromosomal rearrangements. The very fact that, in mammals, duplicated genes of more ancient origin are found unlinked

from each other was the first clue which made us suspect that a mammalian ancestor might have gone through at least one round of tetraploid evolution at the stage of fish. The genes for isozymic forms of various enzymes are often not linked to each other. Similarly, the haemoglobin α -chain gene is not linked to the β , γ , δ -chain gene cluster, and immunoglobulin light-chain genes are not linked to the heavy-chain gene cluster.

Tandem Repeats

The argument that all the duplicated genes of mammals originally arose as tandem repeats and that their non-linkage, if found, is merely the consequence of subsequent chromosomal rearrangements is unsound. Tandem repeats form a very close cluster and the chance of an evolutionary compatible chromosome rearrangement breaking up such a tight linkage is as small as that of bringing together two originally unlinked duplicates to form a tight linkage.

Indeed, tandem repeats seem to have stayed as tandem repeats throughout vertebrate evolution. Close linkage of immunoglobulin heavy-chain genes has been observed on all three mammalian species in which sufficient genetic information has been obtained; man, mice and rabbits⁵. Sexually mature testes of mammals as well as of birds contain a specific lactate dehydrogenase subunit; LDH C. Thus a gene duplication which produced LDH C gene must have occurred before the ancestral vertebrate stock diverged into mammalian and avian lines. Yet the genetic information available in certain avian species revealed persistent tight linkage between LDH B and C genes²².

Fish and Amphibians

With the discovery of tetraploid and even octaploid bisexual frog species in South America²³, there was no longer a question that polyploid evolution was possible at the stage of fish and amphibians. Furthermore, on trout and salmon which we consider tetraploid species on the verge of completing the process of diploidization, we found evidence of either doubling of the gene loci (four original homologues already differentiated into two separate pairs) or tetrasomic inheritance (four homologues still forming a quadrivalent in meiosis) with regard to almost all the enzymes and proteins which we and others studied^{4,5}. At this early stage following tetraploid evolution, only a few of the duplicated gene loci would have become silenced by sustaining function-depriving mutations. It is not surprising then to find that trout and salmon appeared to have twice more gene loci for most enzymes even when compared with mammals.

Chromosomal Changes

Regardless of what happened to the fish ancestor of mammals of 300 m.y. ago, at first glance it seems absurd even to entertain the notion that some of the original autosomal linkage groups of this ancestral tetraploid fish might have persisted in recognizable form all the way up to man, so that homologous relationships can still be found among certain pairs of nonhomologous human autosomes. It is well to

remember, however, that proteins and enzymes have changed only slightly during a comparable time.

Of various types of chromosomal rearrangements, reciprocal translocations are the real culprits which break up the linkage groups. Yet they are the very type which seldom accompany successful speciation, simply because heterozygotes for reciprocal translocations are, as a rule, semi-sterile. A near sterile mating between semi-sterile heterozygotes does not often produce the translocation homozygotes necessary for the fixation of a reciprocal translocation as a species characteristic.

The most common chromosomal changes seen in vertebrate evolution are Robertsonian fusions which create one metacentric from two acrocentrics and inversions which, if pericentric in nature, change the position of a centromere. Neither of these changes alone can drastically alter original linkage groups. It is, then, not altogether unreasonable to expect that, in spite of apparently extensive chromosomal rearrangements which occurred since the time of a tetraploid ancestor, homoeologous relationships may still be found among certain pairs of mammalian autosomes in a haploid set.

Chromosome Number

Although the diploid chromosome numbers of mammals range from a low of six to a high of eighty-four, the last census taken by Matthey in 1968 shows that more than half of the 668 species surveyed had numbers between forty and fifty-six; a peak being found at forty-eight²⁴. It may be that a common ancestor of mammals had forty-eight chromosomes. If so, man and other higher primates might represent a group in which the ancestral chromosome complement was conserved with the least amount of chromosomal rearrangements.

Based on banding patterns revealed by quinacrine mustard staining, Comings²⁵ was able to arrange twenty-two autosomes of man into eleven homoeologous pairs. Only chromosome 18 was found to have no apparent partner. Some of these resemblances between nonhomologues may be more apparent than real. But a few are rather convincing. One of these is a pair made of chromosomes 11 and 12 (Fig. 1b and c). Furthermore, by the use of human-rodent somatic cell hybrids, it has been shown that these two chromosomes indeed harbour pairs of isozyme genes for lactate dehydrogenase (LDH)^{26,27} and glutamic-pyruvic transaminase (GPT)²⁷; LDH A gene and GPT C gene are found on chromosome 11, whereas LDH B gene and GPT B gene are found on chromosome 12. The homoeologous relationship seems rather convincing on this pair of human autosomes. My view is that in most instances homoeologous relationships will be found with regard to a small segment rather than along the entire length of two nonhomologues. Using the method of *in situ* mRNA-DNA hybridization, Price *et al.*²⁸ obtained suggestive evidence that the haemoglobin β , γ , δ chain gene cluster is carried by either chromosome 4 or 5, while the haemoglobin α -chain gene is on chromosome 2. Earlier linkage data, however, indicated that the β , γ , δ gene cluster is on chromosome 1 (ref. 29). Chromosomes 1 and 2 were regarded as homoeologous by Comings²⁵. If the β , γ , δ gene complex is carried by chromosome 1 and the α gene by chromosome 2, at least a partial homoeologous relationship between these two largest autosomes of man may eventually be proven. I find, however, a closer resemblance in the banding patterns on the long arms of chromosomes 2 and 4. The exact location of the haemoglobin β , γ , δ chain gene cluster is awaited with great interest.

The idea that meaningful genetic difference which separates man from apes or for that matter man from the mouse or the

horse is really very small might be quite disconcerting to some. But such seems to be the nature of evolution dictated by natural selection which more often than not extols the virtue of *status quo*.

Finally, it should be pointed out that when rapid evolutionary changes are encountered on certain genes, functional triviality of these genes rather than adaptive changes are indicated. Of all the sequenced peptide chains, fibrinopeptides A and B show the fastest rate of evolutionary change; 60 PAM/100 amino acid residues/100 m.y. This rate is 1,000 times faster than that for histone IV (ref. 11). These amino end peptides are discarded from fibrinogen during blood clot formation. Their role being passive, most mutations do not affect the performance of their trivial function; hence, exceptionally rapid changes.

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- ¹ Ohno, S., Becak, W., and Becak, M. L., *Chromosoma*, **15**, 14 (1964).
- ² Ohno, S., *Sex Chromosomes and Sex-linked Genes* (edit. by Labhart, A., Mann, T., and Samuels, L. T.), Monographs on Endocrinology, **1** (Springer-Verlag, Heidelberg, 1967).
- ³ Ohno, S., *A. Rev. Genet.*, **3**, 495 (1969).
- ⁴ Ohno, S., Wolf, U., and Atkin, N. B., *Hereditas*, **59**, 169 (1968).
- ⁵ Ohno, S., *Evolution by Gene Duplication* (Springer-Verlag, Heidelberg, 1970).
- ⁶ Ohno, S., in *Perspectives in Cytogenetics, The Next Decade* (edit. by Wright, S. W., Crandall, B. F., and Boyer, L.), 37 (Charles C. Thomas, Springfield, Illinois, 1972).
- ⁷ Morris, D., *The Naked Ape* (The Trinity Press, London, 1967).
- ⁸ Haldane, J. B. S., *J. Genet.*, **55**, 511 (1957).
- ⁹ Harris, H., *Proc. R. Soc., B*, **164**, 298 (1966).
- ¹⁰ Kimura, M., and Ohta, T., *Nature*, **229**, 467 (1971).
- ¹¹ *Atlas of Protein Sequence and Structure* (edit. by Dayhoff, M. O.), National Biomedical Research Foundation (Silver Springs, Maryland, 1972).
- ¹² Goodman, M., Barnabas, J., Matsuda, G., and Moore, G. W., *Nature*, **233**, 604 (1971).
- ¹³ Crick, F. H. C., *J. Molec. Biol.*, **38**, 367 (1968).
- ¹⁴ Lyon, M. F., *Nature*, **190**, 372 (1961).
- ¹⁵ Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell Res.*, **58**, 128 (1969).
- ¹⁶ Proc. Paris Conf., *Standardization in Human Cytogenetics* (1972).
- ¹⁷ Pearson, P., in *Modern Aspects of Cytogenetics: Constitutive Heterochromatin in Man* (Hoechst Symposium Proceedings, in the press).
- ¹⁸ Deys, B. F., thesis, Aan de Rijksuniversiteit te Leiden (1972).
- ¹⁹ Kimura, M., *Nature*, **217**, 624 (1968).
- ²⁰ Ohno, S., *Nature*, **234**, 134 (1971).
- ²¹ Crick, F., *Nature*, **234**, 25 (1971).
- ²² Zinkham, W. H., and Isensee, H., *Science, N.Y.*, **164**, 185 (1969).
- ²³ Becak, M. L., Becak, W., and Rabello, M. N., *Chromosoma*, **19**, 188 (1966).
- ²⁴ Matthey, R., *Mammalian Chromosomes Newsletter*, **9**, 17 (1968).
- ²⁵ Comings, D. E., *Nature*, **238**, 455 (1972).
- ²⁶ Ruddle, F. H., Chapman, V. M., Chen, T. R., and Klebe, R. J., *Nature*, **227**, 251 (1970).
- ²⁷ van Someren, H., Meera Khan, P., Westerveld, A., and Bootsma, D., *Nature* (in the press).
- ²⁸ Price, P. M., Conover, J. H., and Hirschhorn, K., *Nature*, **237**, 340 (1972).
- ²⁹ Nance, W. E., Conneally, M., Kang, K., Reed, T., Schroder, J., and Rose, S., *Am. J. hum. Genet.*, **22**, 453 (1970).
- ³⁰ Trujillo, J. M., Walden, B., O'Neil, P., and Anstall, H. B., *Science, N.Y.*, **148**, 1603 (1965).
- ³¹ Mathai, C. K., Ohno, S., and Beutler, E., *Nature*, **210**, 115 (1966).
- ³² Ohno, S., Poole, J., and Gustavsson, I., *Science, N.Y.*, **150**, 1737 (1965).
- ³³ Epstein, C. J., *Science, N.Y.*, **163**, 1078 (1969).
- ³⁴ Richardson, B. J., Czuppon, A. B., and Sharman, G. B., *Nature new Biol.*, **230**, 154 (1971).
- ³⁵ Cooper, D. W., VandeBerg, J. L., Sharman, G. B., and Poole, W. E., *Nature new Biol.*, **230**, 155 (1971).
- ³⁶ Lyon, J. B., *Biochem. Genet.*, **4**, 169 (1970).
- ³⁷ Graham, J. B., Buckwalter, J. A., Hartley, L. J., and Brinkhaus, K. M., *J. exp. Med.*, **90**, 97 (1947).
- ³⁸ Mustard, J. F., Roswell, H. C., Robinson, G. A., Hoeksema, T. D., and Downie, H. G., *Br. J. Haemat.*, **6**, 259 (1960).
- ³⁹ Gavin, J., Noades, J., Tippet, P., Sanger, R., and Racc, R. R., *Nature*, **204**, 1322 (1964).
- ⁴⁰ Drieux, H., Priouzeau, M., Thiery, G., and Priouzeau, M. L., *Recl. Méd. Vét. EC. Alfort*, **126**, 385 (1950).

Absorber Theory of Radiation and the Future of the Universe

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The power input to a microwave source remained constant to 1 part in 10^8 as it alternately radiated into free space and into a local absorber. In the framework of the Wheeler-Feynman absorber theory of radiation, this result sets some constraints on possible future properties of the Universe.

THE existence of complete absorption of radiation along the future light cone is normally taken as a precondition for the application of the Wheeler-Feynman absorber theory^{1,2} (but see ref. 1, page 175, for a counter example). But as Davies has recently been at pains to point out³, many Friedmann models are not opaque in the future. In particular, all open, ever-expanding, cosmological models exhibit less than complete absorption along a future light cone. If our Universe conforms to such a model, one might detect some of the advanced effects referred to in ref. 1. In this article I report the results of a sensitive search for one such effect, a reduced power drain on a source radiating into an incomplete absorber.

It is also possible to link the question of the absorptive properties of the Universe to the oft-debated question of the direction of time's arrow in closed cosmological models. The direction of time may be separately specified by considering cosmology (expansion of the Universe), statistical mechanics (increase in entropy) or the emission of electromagnetic energy. Are these three arrows of time related, and if so, how? This question is discussed at length by Gold^{4,5}, Gal-Or⁶, and Cocke⁷, who presents a model for "two-time statistical boundary conditions", suggesting a symmetrical reversal of time's arrow at the point of maximum expansion of the Universe.

More recently, Davies⁸ raised the issue again, and discussed in some detail an objection to simple models of the type suggested by Gold⁵ and Cocke⁷. One may understand the objection by considering a diverging beam of monochromatic radiation emitted into free space at time A , such as the present, in the expanding phase of a closed Universe (Fig. 1). I assume that some of the radiation passes the epoch of maximum expansion and is absorbed (as far as we are concerned) at B , which need not be near the singularity, $R=0$. In a time reversed cosmology, an "observer" at B will interpret the event as an emission. To produce absorption of the diverging beam originating at A , a very large number of sources at locations like B must spontaneously emit radiation of precisely the

correct phase and frequency to produce a temporal mirror image of the signal emitted at A (my unpublished work with J. A. Wheeler). As Davies argues, this "accidental conspiracy" is unlikely. If the "accidental conspiracy" does not occur in the time reversed model, then radiation emitted at A may not be fully absorbed.

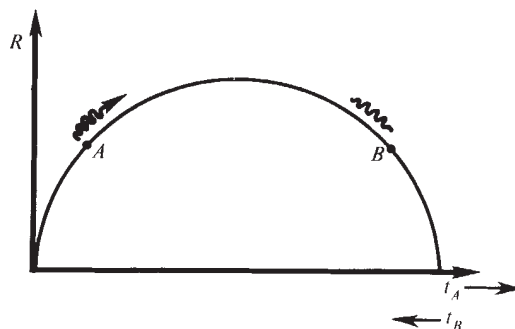


Fig. 1 A time-symmetric universe. An observer at A emits radiation and assumes it is absorbed at B . An observer at B (with a time direction t_B) views this process as an emission.

I have considered two cases where radiation emitted into free space by a properly chosen source may be incompletely absorbed. In the framework of the Wheeler-Feynman theory, incomplete absorption would imply a smaller radiation reaction and therefore a lower power drain on a source when it radiates into free space than when it radiates into a local (complete) absorber:

$$P_f = (1 - \delta)P_a \quad (1)$$

Here P_f is the power input to a source when it radiates into free space and P_a is the power drain when it radiates into a total, local, absorber. Ordinary experience dictates that δ must be small. The aim of the experiment reported here was to set a direct upper limit on δ .

Cosmological Absorption of Microwave Radiation

Can we transmit an electromagnetic signal which, if it is absorbed at all, will be absorbed only at very large distances,

in the very distant future? The answer seems to be yes. I chose to work with microwaves because the absorbing properties of local matter at microwave frequencies are known to be small. The absorption of the Earth's atmosphere at $\lambda = 3$ cm is less than 3% at the zenith in clear weather. Absorption of 3 cm radiation by the Galaxy is negligible except at low galactic latitudes. In addition, the antenna used had a beam far larger than the angle subtended by the galactic plane. Hence, I can safely set galactic absorption $\leq 1\%$.

Intergalactic extinction of two types may occur. First, there may be a line absorption by neutral intergalactic hydrogen. As the 3 cm radiation propagates through an expanding Universe it will be redshifted. If the Universe continues to expand sufficiently, the wavelength of the radiation relative to the matter it traverses will reach 21 cm, so that it will be absorbed by neutral hydrogen. It is easy to show, however, that this absorption can be neglected. I make the extreme assumption that all the matter in the Universe is in the form of neutral hydrogen. Observed upper limits⁹ on the deceleration parameter, $q_0 \leq 1.0$, imply that the present density of hydrogen is $\leq 3.5 \times 10^{-29} \text{ g cm}^{-3}$ for $H_0 \leq 100$. At the epoch in the future when 3 cm radiation has been redshifted by a factor $z+1 \simeq 7$, the density of neutral hydrogen will be $\leq 10^{-31} \text{ g cm}^{-3}$. The work of Field¹⁰ may be used to show that the 21 cm line absorption is less than 1.5×10^{-3} , where I have used the fact that the spin temperature T_s must be at least as large as the temperature of the microwave background at that epoch, $2.7 (z+1)^{-1}$.

Table 1 Values of $\Delta I/I$, Determined from the Chart Recordings, for Four Phases of the Signal Relative to the Cosine Wave Driving the Ferrite Switch

Phase (ϕ)	Termination at AA'	No. of runs	$\Delta I/I (\times 10^4)$
0°	Absorber	38	$+14.7 \pm 7.7$
90°	Absorber	26	-6.4 ± 7.4
180°	Absorber	40	-10.3 ± 7.7
270°	Absorber	24	-8.0 ± 8.8
0°	Antenna	14	-7.4 ± 7.7
180°	Antenna	17	0.0 ± 5.6

In each case, ΔI is the difference in the input current with the principal horn antenna uncovered and covered: $\Delta I = I_u - I_c$. Errors are standard deviations of the means.

Second, Thomson scattering from intergalactic electrons may occur¹¹. The mean free path against scattering is very large, $\gtrsim cH_0^{-1}$, and in any case, scattering, unlike absorption, is a time-symmetric process.

I conclude that at least 95% of the microwave radiation leaving the antenna will reach cosmological distances before being absorbed (if it is ever absorbed^{3,12}).

Experimental Details

The apparatus used in these observations is shown schematically in Fig. 2. Microwaves at 9.7 GHz were generated by a commercial impatt diode oscillator (Varian model VSX 9522P). The efficiency, ϵ , of this diode was about 4% at the voltage level used. The output of the oscillator was alternately switched between an absorptive termination and a large conical horn antenna (originally constructed by Roll and Wilkinson¹³). The switching element was a four-port ferrite switch driven at 1 kHz: the insertion loss at 9.7 GHz was a few tenths of a dB. During each cycle of the ferrite switch, the diode thus radiated alternately into a virtually complete "here and now"

absorber and into the antenna which was pointed at the zenith. Had the radiation reaction caused by local, and by very distant, matter differed, the power inputs to the diode would have changed at the switching frequency and with a known phase. The power input to the microwave oscillator was monitored by measuring the current supplied to the impatt diode by a constant voltage power supply (Hewlett Packard model 6106A). Only the time varying (differential) power consumption is of interest. Therefore, a.c. preamplification and detection were used throughout.

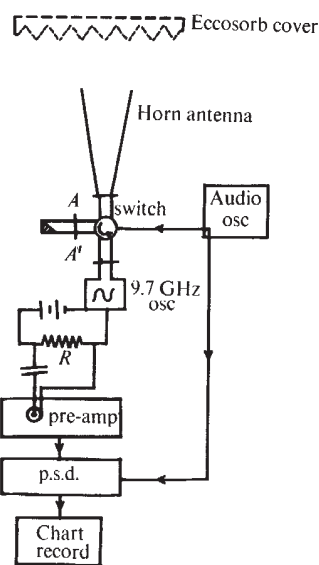


Fig. 2 Schematic of the apparatus. When in place, the eccosorb cover was actually 20λ above the opening of the antenna. For some observations, the absorptive termination at AA' was replaced by another small horn antenna radiating into free space.

Before phase sensitive detection, any 1 kHz voltage drop across resistor R was amplified 1,000 times by a low noise PAR model 113 preamplifier, with a 3 dB roll-off set at 300 and 3,000 Hz. Four phase settings were used on the phase sensitive detector: one with the signal input nominally in phase with the 1 kHz switching voltage, and others at 90°, 180° and 270° to it. The d.c. output of the phase sensitive detector was smoothed with a time constant of 1 s and recorded. Data were taken in 2 min blocks for each phase setting. Calibration of the phase sensitive detector was obtained by introducing a 1 kHz signal with known phase and voltage to its input.

To avoid systematic errors introduced by offsets in the phase sensitive detector and other electronics, alternate runs were made (1) with the antenna radiating freely into space; and (2) with the antenna radiating into a large absorbing sheet placed above the antenna. In the latter case, the output of the oscillator was at all times completely absorbed "here and now" for either position of the ferrite switch. Comparing runs with and without the local absorber above the antenna allows one to eliminate the systematic errors introduced by the electronics.

The absorbing cover was constructed of Emerson and Cuming CV-4 eccosorb, which has a reflexion coefficient less than -40 dB at 9.7 GHz. To ensure that near-field effects were minimized, the absorbing cover was placed twenty wavelengths above the antenna horn opening. Enough eccosorb was used to cover the antenna beam down to the -25 dB level in the E plane of the horn and to the -33 dB

level in the H plane. Direct measurements of the antenna pattern¹⁴ show that the absorption was 99% or more complete with the cover in place.

Five separate sets of observations were made, each including many runs with the antenna covered and many with the antenna uncovered. Clear, cloudless days and nights in August and September were chosen; runs were not made when the galactic plane lay near the zenith.

Finally, towards the end of the experiment, a further check on systematic error was made by replacing the absorptive termination at AA' (see Fig. 2) with a small horn antenna radiating freely into space. With an antenna on each side of the ferrite switch, the radiometer was thus balanced when the eccosorb cover was absent. In this configuration, runs were made with only two phase settings, 0° and 180° .

Results of the Experiments

The chart records provided values for ΔI , the difference in the current inputs with the antenna uncovered and covered. These values are tabulated below for each of the four phase settings employed. If δ is greater than 0 (see equation (1)), then $\Delta I/I$ should be negative for $\phi=0^\circ$ and positive for $\phi=180^\circ$.

Two additional steps are required to convert values for $\Delta I/I$ into a value (or upper limit) for δ . First, it is clear that ΔI will be proportional to the change in the power input only if the voltage across the impatt diode oscillator is constant. This was checked by monitoring the applied voltage directly; the proportional variation in the voltage at the switching frequency $\Delta V/V$ was always $\lesssim 4 \times 10^{-11}$ which is less than the measured values of $\Delta I/I$, and may be neglected. Next, I must take into account the efficiency of the diode. If the total power consumption is P_c when the diode radiates into a local absorber,

$$P_c = P_h + \varepsilon P_e$$

where εP_e is the part of P_c actually emerging as microwave radiation and the remaining power (P_h) is dissipated as heat. The purpose of the experiment was to discover whether the power input to the diode changed when it was allowed to radiate into free space (antenna uncovered). I assume that only the part of the input power which actually emerges as microwaves is affected. Then

$$P_u = P_h + \varepsilon P_u = P_h + \varepsilon(1 - \delta)P_c$$

and

$$\Delta P \equiv P_u - P_c = -\varepsilon \delta P_c$$

hence

$$\delta = -\Delta P / \varepsilon P_c = -\Delta I / \varepsilon I$$

where ε was experimentally determined to be $(3.8 \pm 0.2)\%$ during the observations.

Figure 3 is a plot of δ calculated in this fashion as a function of the phase setting, ϕ , of the detector. The effects predicted in the first section, with $\delta > 0$, should have produced a cosine wave with a maximum at zero phase. A cosine curve was least-squares fit to the four points of Fig. 3, with the further restriction that it should have a maximum at $\phi=0^\circ$.

The result, and its associated standard deviation of the mean, is

$$\delta = (-1.1 \pm 1.6) \times 10^{-9} \quad (2)$$

A negative value of δ , if significant, would have implied that it

"costs" more power to radiate into free space than into a complete, local, absorber. Even if we treat both phase and amplitude as free parameters in the fitting procedure, δ is clearly less than a few parts in 10^9 .

The result is consistent with $\delta=0$, and thus conforms to ordinary expectations. It seems either that the analysis in terms of the Wheeler-Feynman absorber theory is incorrect or that absorption along the future light cone is complete to better than one part in 10^8 .

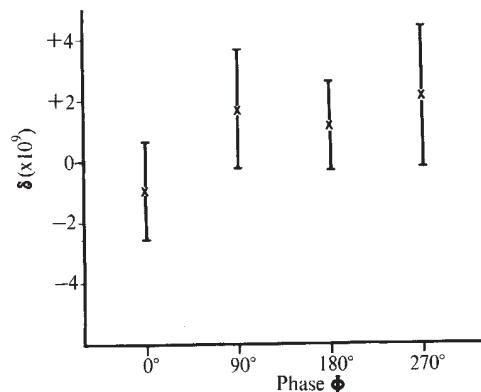


Fig. 3 The quantity δ as a function of the phase of the second detector. All runs have been combined, weighted by the inverses of the respective standard deviations of the means.

Finally, T. Gold (private communication) has pointed out that the existence of advanced effects, such as those searched for unsuccessfully here, might have vitiated the usual *primaevae* fireball explanation of the isotropic microwave background radiation.

Although the results of the experiment are not startling, like other null results (the Eötvös experiment^{14,15} and the Hughes-Drever experiment^{16,17}), they may prove useful to those investigating the general question of the interaction between local and cosmological physics.

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- ¹ Wheeler, J. A., and Feynman, R. P., *Rev. Mod. Phys.*, **17**, 157 (1945).
- ² Wheeler, J. A., and Feynman, R. P., *Rev. Mod. Phys.*, **21**, 425 (1949).
- ³ Davies, P. C. W., *J. Phys.*, A, **5**, 1722 (1972).
- ⁴ Gold, T., *Am. J. Phys.*, **30**, 403 (1962).
- ⁵ Gold, T., *The Nature of Time* (Cornell University Press, Ithaca, New York, 1967).
- ⁶ Gal-Or, B., *Science*, **176**, 11 (1972).
- ⁷ Cocke, W. J., *Phys. Rev.*, **160**, 1165 (1967).
- ⁸ Davies, P. C. W., *Nature phys. Sci.*, **240**, 3 (1972).
- ⁹ Sandage, A., *Astrophys. J.*, **178**, 1 (1972).
- ¹⁰ Field, G. B., *Astrophys. J.*, **129**, 525 (1959).
- ¹¹ Bahcall, J. N., and Salpeter, E. E., *Astrophys. J.*, **142**, 1677 (1965).
- ¹² Hoyle, F., and Narlikar, J. V., *Proc. R. Soc.*, A, **277**, 1 (1964).
- ¹³ Roll, P. G., and Wilkinson, D. T., *Ann. Phys.*, **44**, 289 (1967).
- ¹⁴ Roll, P. G., *et al.*, *Ann. Phys.*, **26**, 442 (1964).
- ¹⁵ Braginsky, V., and Panov, V., *Zh. Eksp. Teor. Fiz.*, **61**, 873 (1971).
- ¹⁶ Hughes, V., *et al.*, *Phys. Rev. Lett.*, **4**, 342 (1960).
- ¹⁷ Drever, R., *Phil. Mag.*, **6**, 683 (1961).

Horizontal Transmission of Feline Leukaemia Virus

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As feline leukaemia virus (FeLV) is shown to be transmitted horizontally it may be possible to vaccinate cats against lymphosarcoma and other FeLV-associated diseases.

MOST information on the spread of the leukaemogenic RNA viruses under laboratory and field conditions comes from the study of these viruses in the mouse and chicken. Vertical transmission (transmission of virus from parent to progeny through the gametes) is assumed to be the predominant route for the naturally occurring leukaemia viruses of the mouse^{1,2}. Considerable evidence indicates that these viruses, in mice as well as fowl, are integrated in the host genome and are transmitted as Mendelian traits^{3,4}. Horizontal transmission (viral spread by contact or contagion) is thought to play little or no part in the natural history of the murine leukaemia viruses. In fowl, however, contact infection has been convincingly demonstrated, showing that vertical and horizontal transmission need not be mutually exclusive⁵.

The discovery of leukaemia virus in cats⁶ and the development of serological methods⁷⁻¹⁰ for identifying the feline leukaemia virus (FeLV) allow the determination of the relative importance of these two modes of transmission in animals other than mice and fowl. Serological studies have established that FeLV can be demonstrated in approximately 90% of cats with feline lymphosarcoma. In addition to lymphosarcoma, other feline diseases have shown a striking association with FeLV. These include myeloproliferative disorders, infectious peritonitis, fibrosarcomas, and certain non-responsive anaemias⁷⁻⁹. Furthermore, as in the mouse and chicken, clinically normal cats have been found to harbour FeLV⁹.

Recent epidemiological studies on feline lymphosarcoma gave no evidence for horizontal transmission of FeLV¹¹⁻¹³ but cases of apparent contact infections with FeLV have been observed in laboratory cat colonies^{14,15}. We have previously reported the occurrence of numerous household

clusters of feline lymphosarcoma (two or more cases in cats from one household^{7,9,16}). Most of the cluster cases occurred in unrelated cats, a fact that suggested horizontal transmission of FeLV, with subsequent development of lymphosarcoma. The use of a simple serological test for the detection of FeLV has now made possible the testing of a large population of clinically normal cats. Our findings indicate that horizontal transmission of FeLV is common and that cats naturally infected in this fashion have a high risk of developing lymphosarcoma.

Occurrence in Normal Cats

Peripheral blood smears from cats were tested for FeLV by indirect immunofluorescence using absorbed rabbit anti-serum to FeLV group specific antigens⁹. Figure 1 illustrates the characteristic cytoplasmic fluorescence seen in the leukocytes and platelets of FeLV infected cats. Table 1 shows the results of parallel tests on specimens using three methods for FeLV detection; the immunofluorescence on peripheral

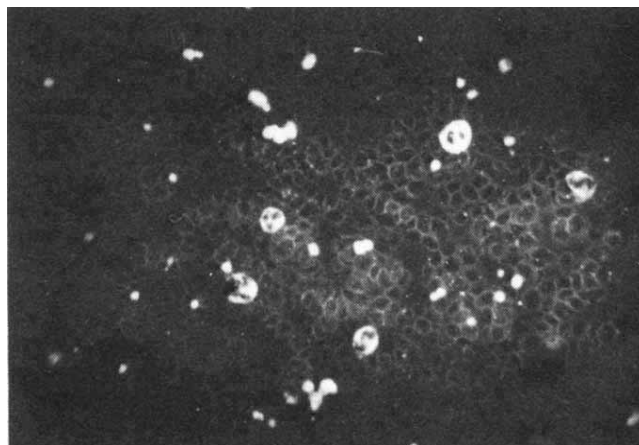


Fig. 1 Punctate granular cytoplasmic fluorescence representing FeLV gs antigen in peripheral blood leukocytes and platelets from a normal cat living in a household with a history of feline lymphosarcoma.

blood smears⁹, the detection of FeLV group specific antigens by immunodiffusion⁷ and the recovery of infectious FeLV in tissue culture⁸. The methods all gave similar results, indicating that demonstration of FeLV group specific antigens in peripheral blood cells by immunofluorescence identifies FeLV infected cats.

Thirty-eight household clusters of feline lymphosarcoma were studied (Table 2). One hundred and seventeen cats developed lymphosarcoma in the cluster households (average of 3.1 cats with lymphosarcoma per household), two-thirds of the cases occurring in unrelated (not parent, offspring or sibling) cats (Table 2).

or other diseases? We have followed up 148 of the 177 FeLV⁺ normal cats identified in our studies (Table 5). 16.2% have developed histologically confirmed lymphosarcoma and 7.5% of the cats have died of non-responsive anaemias with no histological evidence of lymphosarcoma. The average time elapsing between FeLV detection and lymphosarcoma development was 5.3 months, with a range of 1 to 24 months. The expected lymphosarcoma incidence in the general cat population for a 5.3 month period is 18.3 cases per 100,000 cats at risk¹⁷, thus we would have expected 0.018 cases to have developed in these 148 cats. The observed incidence of lymphosarcoma in FeLV⁺

Table 1 Comparison of FeLV Detection Methods

Diagnosis	Number of cats	FeLV status determined by		Infectious FeLV isolated in tissue culture from			
		Immunofluorescence	Immunodiffusion	Tumour	Plasma	Salivary gland	Urine
Lymphosarcoma	5	+	+	5	5	5	3
Normal	5	+	+	No tumour	5	5	2
Normal	5	—	—	No tumour	0	0	0

Tumour tissue and salivary glands were extracted as 20% homogenates in phosphate buffered saline. The extracts (clarified by centrifugation), undiluted plasma and undiluted urine were filtered through a 220 µm filter and 1 ml of the filtrate was added to freshly passed cultures of feline embryo fibroblasts (FEF). FeLV infection was assayed by development of FeLV gs antigen (demonstrated by immunofluorescence⁹) in acetone fixed FEF cells or by isolating FeLV from culture media by density gradient centrifugation and demonstrating FeLV gs antigen by immunodiffusion⁷.

Table 3 shows the results of immunofluorescence tests on the peripheral blood of 543 apparently normal cats from households with a history of cats with lymphosarcoma or other FeLV-associated diseases. 33% of these normal animals were infected with FeLV. Normal cats living in households already having two or more cats with lymphosarcoma were twice as likely to be infected with FeLV than those from households having only one cat with lymphosarcoma or cats with other FeLV-associated diseases.

normal cats was 888 times greater than the expected incidence.

The natural development of lymphosarcoma was followed radiographically in a normal cat shown to be infected with FeLV. Initial radiographs of the cat showed no evidence of lymphosarcoma (Fig. 2a). Three months later, radiographs of the thoracic cavity revealed a large lymphoid tumour mass of the anterior mediastinum (Fig. 2b).

A syndrome of FeLV-associated non-responsive anaemias

Table 2 Kinship and FeLV Status of Cats with Lymphosarcoma from Cluster Households

Total number of clusters	Number of cats with lymphosarcoma			FeLV status of cats with lymphosarcoma		
	Total	Unrelated cats	Related cats	Number examined	FeLV ⁺	FeLV ⁺ (%)
38	117	78	39	56*	51	91

Cluster household cats were considered related if lymphosarcoma developed in either parent and offspring, or two or more siblings. FeLV status was determined by immunofluorescent detection of FeLV gs antigen in leukocytes and platelets in peripheral blood smears.

* Only fifty-six of the 117 cats that developed lymphosarcoma were examined for FeLV. The remaining sixty-one cats died before the study began.

Table 4 summarizes our experience with immunofluorescence tests on 2,005 apparently normal cats, 543 living in households with a known history of an FeLV-associated disease and 1,462 living under other circumstances. Normal cats living with infected cats have a high risk of becoming infected. Overt FeLV infection in normal cats living under other circumstances is extremely rare. Thus only two of 1,462 (0.14%) normal cats from environments other than households with a history of FeLV-associated diseases were infected with FeLV.

FeLV-Related Disease in Carriers

What is the subsequent course of FeLV⁺ normal cats? Do they have a heightened incidence of lymphosarcoma

in cats has been recognized and appears similar to the disease induced in Balb/c mice by certain strains of Rauscher leukaemia virus (ref. 18 and W. D. H., jun., unpublished). Epidemiologists looking only for lymphosarcoma as evidence for horizontal infection with FeLV have not included deaths due to anaemia in their data. If we combine the FeLV⁺ normal cats that developed lymphosarcoma and those that developed non-responsive anaemias, then a total of 23.7% of all FeLV⁺ normal cats developed an FeLV-related disease. In this connexion, many young cats die of an ill defined syndrome known as the "fading kitten syndrome". Recent experiments on kittens experimentally infected with FeLV have produced a very similar syndrome which is associated with thymic atrophy¹⁹. Thus, FeLV may be the aetiological agent for

at least two, and possibly more, non-neoplastic diseases of cats.

FelV Transmission

How is FeLV transmitted from cat to cat? Three routes should be considered: saliva, urine, and blood. FeLV was found in the parotid salivary glands of thirty-two FeLV⁺ lymphosarcoma cats but was not found in salivary glands of six FeLV-lymphosarcoma cats nor in eleven normal FeLV⁻ cats. Saliva may be the most likely vehicle for spreading FeLV as cats are social animals using their suitably bristly tongues for grooming themselves and

infestation is very high in the cat population, especially in households with several cats. A hamster reticulum cell sarcoma has been transmitted through the mosquito by transfer of tumour cells^{21,22}. We were able to recover FeLV⁺ leukocytes from a mosquito that sucked blood from an anaesthetized FeLV⁺ cat with lymphosarcoma. Other cat parasites to be considered as possible vectors are nematodes, coccidia, and rickettsia. It is also quite possible that therapeutic blood transfusions of FeLV⁺ blood to FeLV⁻ anaemic cats may account for occasional iatrogenic horizontal transmission of FeLV with the attendant high risk of developing lymphosarcoma. In support of this, we have discovered two FeLV⁺ normal cats that have repeatedly

Table 3 Detection of FeLV in Normal Cats from Households with a History of FeLV Associated Diseases

	Number of households in which normal cats were examined	Number of households with FeLV ⁺ normal cats	Households with FeLV ⁺ normal cats (%)	Number of normal cats tested	Number of FeLV ⁺ normal cats	FeLV ⁺ normal cats (%)
Households with a history of feline lymphosarcoma	78	44	56.4	395	154	38.9
Non-cluster households (one case of lymphosarcoma)	50	22	44.0	118	33	19.5
Cluster households (two or more cases of lymphosarcoma)	28*	22	78.5	277	121	43.6
Households with FeLV-associated diseases other than lymphosarcoma	25	8	32.0	148	23	15.5
FeLV-associated disease households Total	103	52	50.5	543	177	32.6

* Twenty-eight of the thirty-eight lymphosarcoma cluster households (see text) had clinically normal cats.

Table 4 Detection of FeLV in Normal Cats and Frequency of FeLV Infection Correlated with Exposure to Cats with FeLV-Associated Disease

Environment of normal cats	History of FeLV-associated disease in household	Number studied	Number of households with FeLV ⁺ normal cats	Number of normal cats tested	Number of FeLV ⁺ normal cats
Multiple cat households	Yes	103	52	543	177
Multiple cat households	No	47	0	130	0
Single cat households	No	497	0	497	0
Stray cats	—	638	—	638	2*
Experimental colonies	—	4	0	197†	0
			Total	2,005	179

* Cats used repeatedly as blood donors.

† Including fifty-four cats from a colony receiving chronic administration of ⁹⁰Sr.

companion cats. Aerosolization of FeLV through salivary and nasal secretions may play an important role in disseminating the virus. In support of this it may be noted that Rauscher murine leukaemia virus can be experimentally transmitted to Balb/c mice by exposure to aerosol virus²⁰.

FeLV group-specific antigen can be detected in the kidneys of 70% of cats with lymphosarcoma^{8,9}. Antigen was present in the infiltrating neoplastic lymphocytes as well as in discrete deposits on the glomerular basement membranes. Infectious FeLV and neoplastic cells infected with FeLV can pass through damaged kidneys into the urine (Table 1). The significance of this is evident when one considers the use of communal litter pans in urban households with several cats.

As FeLV is found free in the plasma, as well as budding from leukocytes and platelets in the blood of infected cats, it is conceivable that blood sucking parasites of cats might acquire and transmit FeLV. In warm months flea

served as blood donors (see Table 4), but we were unable to identify those cats that received their viraemic blood.

Implications

Two conclusions emerge from our study. Non-infected cats living in close proximity to cats shedding FeLV have a greatly increased risk of becoming infected and, once infected, the cat has an approximately 900 times greater risk of developing lymphosarcoma.

Whether the exogenous FeLV induces lymphosarcoma, or activates an endogenous virus which does so, is an open question. The important point is that it should be possible to break the cycle of horizontal transmission by vaccination or by selectively segregating or eliminating FeLV⁺ cats from the cat population. We are now attempting to protect cats at high risk by vaccinating them with attenuated FeLV.

As FeLV replicates so well in human cell tissue culture²³ and as cats live in such close proximity to humans, the question of human infection with FeLV is important. Epidemiological evidence concerning this is inconclusive²⁴⁻²⁶. We have not found any antigen related to FeLV in more than 148 specimens of normal or neoplastic human tissues^{7,9} and FeLV antigen has not been found in blood smears

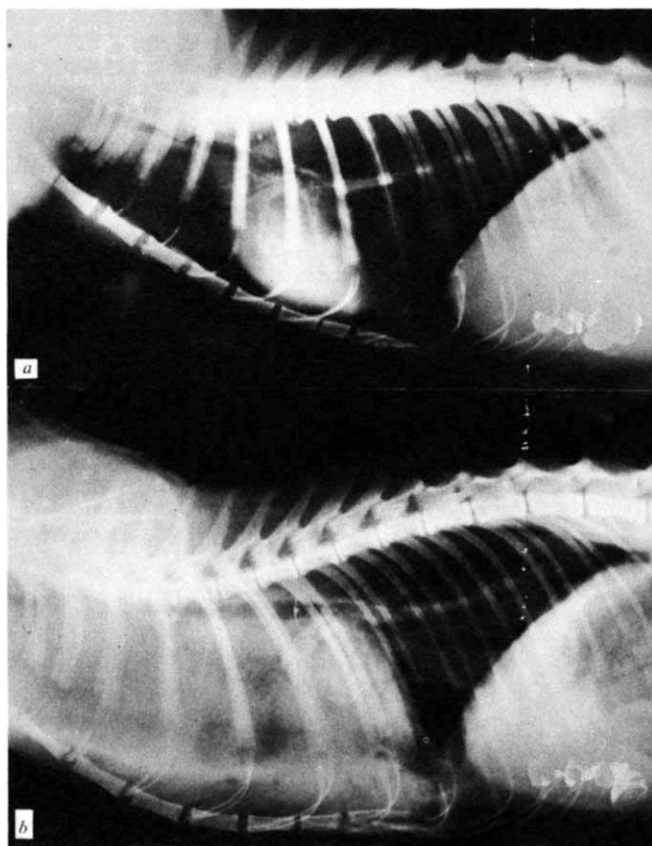


Fig. 2 *a*, Thoracic radiograph of an FeLV⁺ normal cat. There is no evidence of lymphosarcoma. *b*, 3 months later radiographs reveal thoracic effusion and a large anterior mediastinal lymphoid mass.

from 130 humans, including forty-three individuals living in households with FeLV⁺ cats, fifty-seven veterinary surgeons and thirty employees of veterinary hospitals. Much additional evidence needs to be obtained, however, before concluding that human infection with FeLV-neutralizing antibody exists in human serum. We have recently found that some cats are highly resistant to FeLV and have high titres of neutralizing antibody. A similar response to FeLV infection may occur in humans.

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Table 5 Summary of Disease Development in FeLV Infected Normal Cats

Result	Number of cats	Cats followed up (%)
Total FeLV positive normal cats	177	—
Follow ups available	148	100
Alive and well	91	61.5
Death due to		
Lymphosarcoma	24*	16.2
Anaemia	11	7.5
Panleukopaenia	13	8.7
Feline infectious peritonitis	5	3.4
Other	4	2.7
Total	57	38.5
Follow-up not available		
Killed while healthy	14	—
Lost contact with owners	14	—
Cat lost	1	—
Total	29	—

* The average time required to develop lymphosarcoma after detection of FeLV infection was 5.3 months (range 1 to 24 months).

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- ¹ Gross, L., in *Oncogenic Viruses* (Pergamon, New York, 1970).
- ² Huebner, R. J., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1087 (1969).
- ³ Emmelot, P., and Bentvelzen, P., *RNA Viruses and Host Genome in Oncogenesis* (North-Holland, Amsterdam, 1971).
- ⁴ Old, L. J., and Boyse, E. A., *Harvey Lect.*, **67**, 273 (1973).
- ⁵ Rubin, H., Cornelius, A., and Faushier, L., *Proc. natn. Acad. Sci. U.S.A.*, **47**, 1058 (1961).
- ⁶ Jarrett, W. F. H., Crawford, E. M., Marsin, W. B., and Davie, F., *Nature*, **202**, 567 (1954).
- ⁷ Hardy, jun., W. D., Geering, G., Old, L. J., de Harven, E., Brodey, R. S., and McDonough, S., *Science*, **166**, 1019 (1969).
- ⁸ Hardy, jun., W. D., *J. Am. vet. med. Ass.*, **158**, 1060 (1971).
- ⁹ Hardy, jun., W. D., Hirshaut, Y., and Hess, P., *Fifth Int. Symp. Comp. Leukaemia Res., Bibl. Haematol.*, **34**, 778 (Karger, Basel, and New York, in the press).
- ¹⁰ Sarma, P., Baskar, J. F., Huebner, R. J., Old, L. J., and Hardy, jun., W. D., *Proc. Fourth Int. Symp. Comp. Leukaemia* (Karger, Basel, 1970).
- ¹¹ Schneider, R., *J. Am. vet. med. Ass.*, **157**, 1753 (1970).
- ¹² Schneider, R., *J. Am. vet. med. Ass.*, **158**, 1125 (1971).
- ¹³ Schneider, R., *Int. J. Cancer*, **10**, 345 (1972).
- ¹⁴ Rickard, C. G., *J. small Anim. Pract.*, **10**, 615 (1969).
- ¹⁵ Jarrett, W., *J. clin. Pathol.*, **25**, 43 (1972).
- ¹⁶ Brodey, R. S., McDonough, S., Frye, F. L., and Hardy, jun., W. D., *Proc. Fourth Int. Symp. Comp. Leukaemia* (Karger, Basel, 1970).
- ¹⁷ Dorn, C. R., Taylor, D. O. N., and Hibbard, H. H., *Am. J. vet. Res.*, **28**, 993 (1967).
- ¹⁸ Camiscoli, J. F., LoBue, J., Gordon, A. S., Alexander, P., Schultz, E. F., and Weitz-Hamburger, A., *Cancer Res.*, **32**, 2843 (1972).
- ¹⁹ Anderson, L. J., Jarrett, W. F. H., Jarrett, O., and Laird, H. M., *J. natn. Cancer Inst.*, **47**, 807 (1971).
- ²⁰ McKissick, G. E., Griesemer, R. A., and Farrell, R. L., *J. natn. Cancer Inst.*, **45**, 625 (1970).
- ²¹ Banfield, W. G., Woke, P. A., MacKay, C. M., and Cooper, H. L., *Science*, **148**, 1239 (1965).
- ²² Woke, P. A., and Konwinski, N., *J. natn. Cancer Inst.*, **48**, 219 (1972).
- ²³ Jarrett, O., Laird, H. M., and Hay, D., *Nature*, **224**, 1208 (1969).
- ²⁴ Bross, I. D. J., and Gibson, R., *J. Med.*, **1**, 180 (1970).
- ²⁵ Bross, I. D. J., Bertell, R., and Gibson, R., *Am. J. Publ. Hlth.*, **62**, 1520 (1972).
- ²⁶ Schneider, R., *Int. J. Cancer*, **10**, 338 (1972).

LETTERS TO NATURE

PHYSICAL SCIENCES

5 GHz Emission from β Persei

THE triple star system β Persei (Algol) is an erratic radio source¹ which has occasional periods of activity lasting for several days. Jones and Woolf² suggest that the emission results from mass transfer between Algol A and B, the components of the 2.8 d eclipsing binary. Hjellming³ has discussed the parameters of possible models for the emission region.

We report here observations of the source made at 5 GHz during the alignment of the 5-km telescope⁴. These results are of interest not only for the information they provide about Algol itself but because measurements of its position provide a method for determining the coordinate system of the telescope in terms of the position of a fundamental star. The details of the astrometric programme will be reported elsewhere (M. R. and Elsmore, in preparation).

The observations were made between June 1972 and February 1973, and cover about 600 h. On most occasions the flux density was measurable, but it was usually too small for astrometric purposes. Many of the observations lasted for 12 h, but when the nature of the variability became clearer a number of shorter and more frequent observations were made. In this way it was sometimes possible to observe the start of a period of activity and to make more efficient use of the telescope.

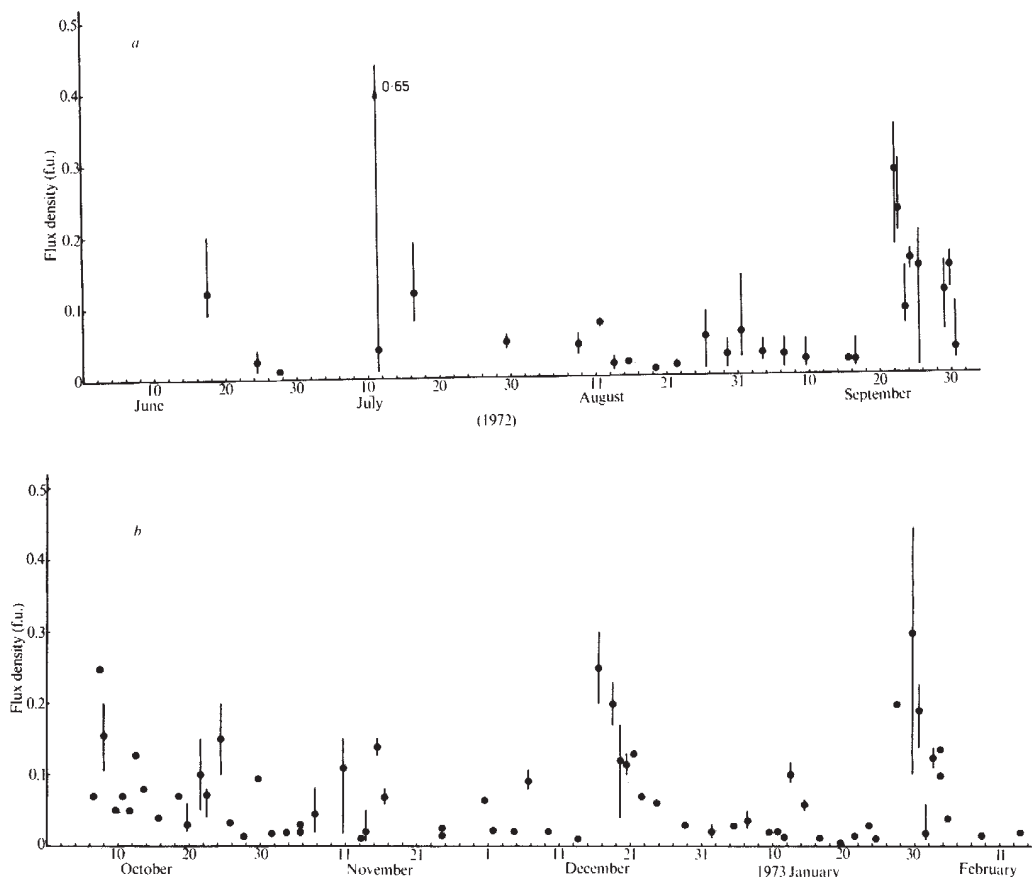
The long-term variation in flux density is shown in Fig. 1. Each point represents the mean value during the observing period of up to 12 h, and in addition the maximum range of flux density is also shown whenever significant variations occurred during the observation. The periods of intense activity last for a few days, and are separated by intervals of many days during which the flux density remains at a few per cent of that during the active periods. The present data show no periodic behaviour in the occurrence of these periods.

Figures 2 and 3 show detailed records of observations when the source was active. The flux densities shown in Fig. 2 are running means over 30 min intervals; no significant variations on shorter time-scales are detectable.

The flare of July 11, 1972, is of particular interest because the flux density attained a value (0.65 f.u.) more than twice that observed so far on any other occasion. Figure 3 shows the outburst with both 5 min and 20 min integration. The flux density for 5 h before and 5 h after the flare was less than 0.02 f.u. The flare itself is almost exactly symmetrical, and has a half-intensity width of 50 min. The tail of this flare was observed by Hjellming, Webster and Balick¹ at 2.7 and 8 GHz. We confirm their measured spectral index of $\alpha \approx 0$ (α is defined by $S \propto \nu^{-\alpha}$) near the start of their observations, but as the flare died away the signal to noise ratio became poor and we cannot confirm their report of a significant increase in α at later times.

It seems necessary to suppose that the radiation is generated

Fig. 1 Flux density of Algol between June 1972 and February 1973. Single values are shown where there was no substantial variation during one observation; otherwise a mean value and overall range are shown.



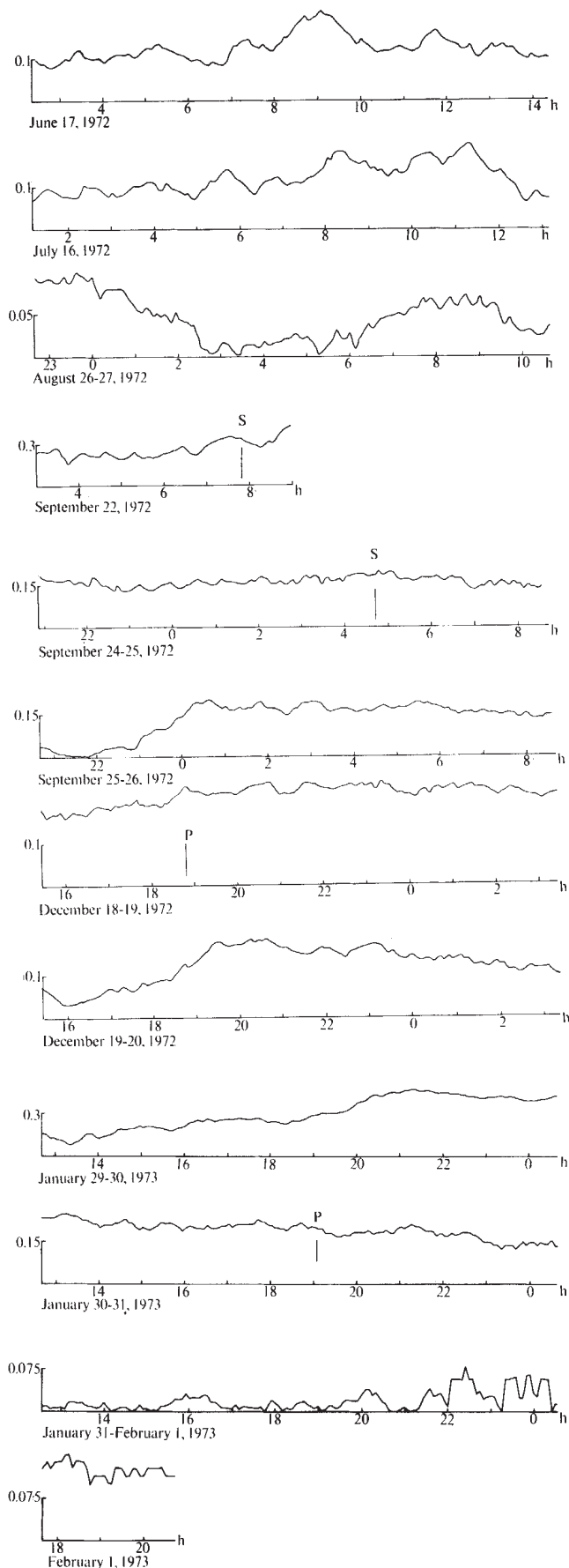


Fig. 2 Variation of flux density (f.u.) of Algol on several days. Each record has been integrated for 30 min.

in the vicinity of Algol AB, as suggested by Hjellming³, or the companion Algol C. In an attempt to distinguish between these two alternatives, the radio position has been measured, relative to the catalogue position, over a period of seven months. Figure 4 shows the means of the measured positions in three intervals of time, compared with the expected orbital positions of Algol AB and C which were computed from the orbital parameters derived by Frieboes-Conde *et al.*⁵ and Hill *et al.*⁶. No account should be taken of the relative zero points of the observed and predicted positions, but the variation of right ascension with time suggests that the emission is indeed associated with Algol AB. Further observations over the whole of the 1.9 yr period are needed to confirm this conclusion.

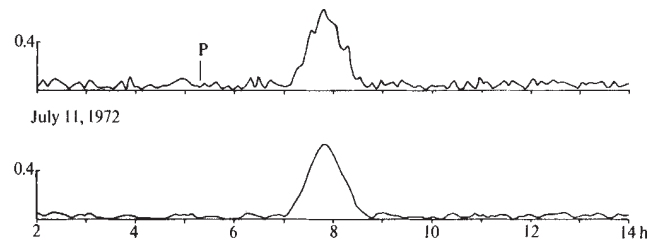


Fig. 3 The event of July 11, 1972; the two versions of the record have integration times 5 min (upper) and 20 min (lower).

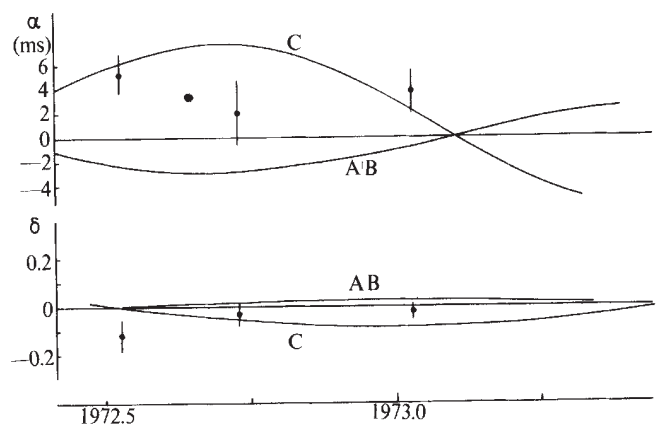


Fig. 4 The position of the radio source relative to the FK4 catalogue position (taking no account of the orbital motion). The curves marked AB and C show the expected orbital positions of Algol AB and C.

Information on the size of the source may be obtained from:

(a) Direct measurement of the angular size. At no time when the source was intense was there any evidence of resolution by the telescope: this sets an upper limit of about 0.7 arc s on the angular size during the active periods. The corresponding limit on the linear size at a distance of 25 pc is 3×10^{12} m, only a little larger than limits set by some of the variations if the excitation is supposed to travel at the speed of light.

(b) Time variations. The most rapid changes occur in about 10 min, implying sizes not more than about 2×10^{11} m for the corresponding emission region, and larger sizes for more slowly varying components. As Hjellming, Webster and Balick¹ point out, however, the physical size of an emission region consisting of thermal plasma is likely to be much smaller, because the excitation may only propagate at the speed of sound. On such a model the size of the flare of July 11, 1972, would have been about 2×10^{10} m, and on most occasions the source would have been less than about 10^{11} m.

(c) Possible occultation phenomena. Occultations of the binary system occur every 2.8 d with a secondary occultation almost symmetrically between the primary occultations. Because the source of energy is presumably associated with either A or B, it is natural to look for significant occultation effects. All

occasions on which occultations occurred during an observation have been examined; some are included in Figs 2 and 3, where the times of the primary and secondary occultations are marked P and S. There is no substantial decrease in the radio flux density associated with either type of occultation. We conclude that the emission region is normally at least 2 or 3 times larger than either Algol A or B (sizes about 2×10^9 m).

The most important restrictions on the size of the source are those set by the time variations ($<10^{10}$ m if heating propagates at the speed of sound, and $<2 \times 10^{11}$ m if heating is by radiation) and by the absence of any significant occultation effects ($\geq 5 \times 10^9$ m). The diameter of the source is therefore fairly closely defined, and is close to the value derived by Hjellming³ for a thermal source at a temperature of 10^8 K and with $N_e = 10^{10}$ cm⁻³, presumably occupying part of the space between Algol A and B. No clear distinction can be made at present between various mechanisms for exciting the plasma.

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¹ Hjellming, R. M., Webster, E., and Balick, B., *Astrophys. J. Lett.*, **178**, L139 (1972).

² Jones, T. W., and Woolf, N. J. *Astrophys. J.*, **179**, 859 (1973).

³ Hjellming, R. M., *Nature*, **238**, 52 (1972).

⁴ Ryle, M., *Nature*, **239**, 435 (1972).

⁵ Frieboes-Conde, H., Herczeg, T., and Høg, E., *Astr. Astrophys.*, **4**, 78 (1970).

⁶ Hill, G., Barnes, J. V., Hutchings, J. B., and Pearce, J. A., *Astrophys. J.*, **168**, 443 (1971).

Effect of Noble Gases on an Atmospheric Greenhouse (Titan)

MEASURED brightness temperatures of Titan in the 8 to 14 μ m range are too high to be produced by simple equilibrium with solar radiation, but at 20 μ m the temperature is more in line with the equilibrium value^{1,2}. An atmospheric "greenhouse" has been invoked to explain these observations, but the amount of hydrogen required to provide the appropriate infrared opacity is much larger than the observed value^{3,4}. Models including large amounts of helium and methane have been considered by Pollack⁵; Hunten⁶ has suggested nitrogen as a possible opacity enhancing agent.

The true composition of Titan's atmosphere remains obscure. Since Kuiper's⁷ discovery of methane, only hydrogen has been added to the list of constituents³. Kuiper's estimate of 200 m amagat for the methane abundance depends on the ambient pressure, which is not known⁸; the derived abundance of hydrogen (5 km amagat) has a quoted uncertainty of about 50% and the observation is tentative³. The presence of other constituents that absorb in the near infrared may be indicated by low resolution observations (ref. 9 and R. L. Younkin, personal communication). Nitrogen and the noble gases would be completely undetectable by present observational techniques.

All previous discussions of the atmospheric opacity problem have ignored the possibility¹⁰ that substantial amounts of neon and argon may be present in the satellite's atmosphere. If the atmosphere is original, captured from the solar nebula, neon could be more abundant than methane. This would result if a large fraction of the original methane were lost as a result of photodissociation and escape of hydrogen with concomitant formation of small amounts of higher hydrocarbons. The latter could contribute to the satellite's reddish colour, either as surface deposits or by forming (or colouring) clouds. The presently existing hydrogen and methane could

then represent an equilibrium mixture which a sufficiently heavy, inert original constituent like neon could now dominate, because its cosmic abundance is only six times less than that of carbon¹¹ and its atomic weight is sufficiently great to prevent thermal escape^{12,13}. Neon seems a more likely constituent than molecular nitrogen (which has a comparable cosmic abundance) because nitrogen should be present as ammonia under conditions of chemical thermodynamic equilibrium^{14,15}. Any original helium would have escaped.

The present atmosphere of Titan may be considered alternatively as the result of outgassing by the satellite. In that case, some ⁴⁰Ar would be released into the atmosphere after production by radioactive decay of ⁴⁰K, as for the Earth. Neon will be present in such a secondary atmosphere too as a result of its adsorption on and occlusion in the accreting material that formed the satellite. This would also be true for helium, but stability against escape is poor. Once again the relative abundances of these gases will depend critically on the subsequent history of the outgassed methane. If much methane (factors of ten) has been lost, neon and/or argon could be major constituents in the present atmosphere.

With these considerations in mind, we have investigated several models for the atmosphere of Titan that include various combinations of neon and argon. Our aim was to see whether the collisional enhancement of the hydrogen opacity would reduce the total amount of hydrogen needed to produce an atmospheric greenhouse effect that was consistent with the infrared temperature measurements. Ideally, the model atmosphere would not require the addition of a cloud layer or some other source of opacity to explain the observations.

The calculation follows that employed by Cess and Khetan¹⁶ in considering H₂ and H₂-He atmospheres for the major planets. We define T_1 as the temperature and P_1 the hydrogen partial pressure at the tropopause, so $T_1^4 = T_e^4/2(1 - 2R/C_p)$ and $P_1^2 = 8/3 Ha_1(C_p/R - 2)$, where T_e is the effective temperature of the satellite and $H = RT_e/g$ is the scale height. The quantity a_1 is determined by fitting an exponential function to the Ne-Ar enhanced hydrogen emissivity which has been evaluated numerically by employing the transition probabilities and rotational line widths of Kiss and Welsh¹⁷. These expressions are valid for Titan providing $a_1HP_2^2 \gg 1$, where P_2 is the hydrogen partial pressure at the surface of the satellite. This condition is satisfied for the model atmospheres we consider. If we now assume that the lapse rate within the troposphere is adiabatic, then $P_2 = P_1(T_2/T_1)^{C_p/R}$.

Table 1 Summary of Greenhouse Calculations for H₂-Ne Atmospheres

Composition Ne/H ₂	a_1 km ⁻¹ atm ⁻²	C_p/R	H km	Surface pressure atm	H ₂ abundance km amagat
0	0.68	2.83	249	0.45	340
5	4.6	2.56	29	2.7	40
10	7.9	2.53	27	3.7	28

These relations illustrate that for a given surface temperature, the hydrogen abundance varies roughly as $\sqrt{H/a_1}$. Hence the addition of large amounts of Ne and/or Ar will substantially reduce the hydrogen abundance required for a given greenhouse effect, because H will be reduced while a_1 is increased because of enhancement of the hydrogen opacity. Results for H₂-Ne mixtures are illustrated in Table 1. These correspond to $T_e = 90$ K, $g = 150$ cm s⁻², and $T_2 = 155$ K, with the latter value selected because of the observed brightness temperatures. The same surface temperature has been inferred⁵ for H₂-CH₄-He atmospheres. Our values of the H₂ abundance and surface pressure for a pure hydrogen atmosphere are in good agreement with Pollack's⁵ more detailed calculations. Calculated brightness temperatures are compared with observed results for H₂-Ne atmospheres in Table 2.

Table 2 Observed and Calculated Brightness Temperatures

Wave-length μm	T_b (observed) K	T_b (calculated) K Ne/H_2 $= 5$	Ne/H_2 $= 10$
8.4	144	143	143
11.0	134	125	126
12.4	125	112	113
20.0	93	97	97

Inclusion of argon reduces the calculated temperatures, which makes H_2 -Ne-Ar atmospheres less attractive than the H_2 -Ne atmospheres. We have not included methane, because Trafton's⁸ interpretation of Kuiper's observations⁷ implies that methane will be a minor constituent if there is a large amount of neon and/or argon present.

The fact that a large amount of neon should be present if the atmosphere is a relic of the solar nebula is an especially attractive feature of these models, because it is hard to justify appropriate abundances of other enhancing agents. As in Pollock's models, however, either a cloud or some additional source of opacity is necessary to reconcile observed and calculated H_2 abundances. There are indications of clouds on Titan¹⁸⁻²⁰ and a layer that has a large optical depth in the visible and near infrared but is transparent in the far infrared could "hide" the hydrogen required for the greenhouse while still permitting the reported infrared temperatures to be measured^{4,5}. With $\text{Ne}/\text{H}_2 = 5$, the cloud-top pressure would be 0.34 atm (assuming the observed 5 km amagat of H_2 is all above the clouds) and this is certainly compatible with the observations.

Clouds themselves would increase the opacity, thus further diminishing the amount of hydrogen required. The composition of such clouds is an open question; methane condensation as proposed by Pollack⁵ may not occur when solar absorption by the ν_3 methane band is taken into account. The hydrocarbon clouds favoured by Sagan⁴ are an attractive possibility, because one certainly expects formation of such substances in this environment. Whatever clouds exist must have a very low reflectivity throughout the visible and near IR if they cover a large percentage of the globe²¹.

Other gaseous absorbers that might relieve the need for more hydrogen include a number of organic molecules (which might actually be present as aerosols rather than vapours) and H_2S .

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- ¹ Allen, D. A., and Murdock, T. L., *Icarus*, **14**, 1 (1971).
- ² Morrison, D., Cruikshank, D. P., and Murphy, R. E., *Astrophys. J. Lett.*, **173**, L143 (1972).
- ³ Trafton, L., *Astrophys. J.*, **175**, 285 (1972).
- ⁴ Sagan, C., *Icarus* (in the press).
- ⁵ Pollack, J. B., *Icarus* (in the press).
- ⁶ Hunt, D., *Comments Astrophys. Space Phys.*, **20**, 149 (1972).
- ⁷ Kuiper, G. P., *Astrophys. J.*, **100**, 378 (1944).
- ⁸ Trafton, L., *Astrophys. J.*, **175**, 295 (1972).
- ⁹ Trafton, L., *Bull. Am. astr. Soc.*, **4**, 367 (1972).
- ¹⁰ Kuiper, G. P., in *The Atmospheres of the Earth and Planets* (edit. by Kuiper, G. P.), ch. 12 (University of Chicago Press, 1952).
- ¹¹ Cameron, A. G. W., in *Origin and Distribution of the Elements* (edit. by Ahrens, L. H.) (Pergamon, Oxford, 1968).
- ¹² Jeans, J., in *Dynamical Theory of Gases*, ch. 15 (Dover, New York 1954).
- ¹³ Spitzer, L., jun., in *The Atmospheres of the Earth and Planets* (edit. by Kuiper, G. P.) (University of Chicago Press, 1952).
- ¹⁴ Greenspan, J. A., and Owen, T., *Science*, **156**, 1489 (1967).
- ¹⁵ Lewis, J. S., *Icarus*, **10**, 393 (1969).

- ¹⁶ Cess, R. D., and Khetan, S., *J. quant. Spectrosc. radiat. Transfer* (in the press).
- ¹⁷ Kiss, Z. J., and Welsh, H. L., *Can. J. Phys.*, **37**, 1249 (1959).
- ¹⁸ Dollfus, A., in *The Solar System*, 3 (edit. by Kuiper, G. P., and Middlehurst, B. M.) (Chicago, 1961).
- ¹⁹ Veverka, J., *Icarus* (in the press).
- ²⁰ Zellner, B., *Icarus* (in the press).
- ²¹ Joyce, R. J., Knacke, R. F., and Owen, T., *Astrophys. J. Lett.* (in the press).

Mercury Concentrations in Dated Varved Marine Sediments collected off Southern California

ANNOUNCEMENTS that mercury concentrations often exceed recommended limits in some oceanic fishes, such as swordfish and tuna, have suggested the possibility that man has polluted the ocean with mercury on a massive scale¹. Although there are examples of localized mercury pollution of certain fresh and coastal waters²⁻⁴, there are few data on historical levels of this metal in the marine environment distant from specific inputs such as wastewater outfalls. Here we report the concentrations of mercury observed in dated varved sediment layers from the Santa Barbara Basin covering the past 150 yr, as well as in two layers estimated to have been deposited approximately 1,500 yr BP. This basin is approximately 100 mile northwest of Los Angeles, and is sufficiently removed from densely populated areas to be protected from the concentrated effects of localized pollutant inputs. The record investigated includes layers deposited before the beginning of the California mining activity in 1849. Thus, it has been possible to compare near surface sediment concentrations of mercury with those observed in layers deposited before man could have released significant amounts of mercury directly into the California Current.

On most of the ocean floor, organisms living in or near the surface sediment stir the bottom material sufficiently to prevent preservation of any short term record, such as annual variations. But in the virtually anoxic Santa Barbara basin off southern California, the lack of active benthic forms has permitted annual variations in the coastal input of sediments to be preserved in an undisturbed sedimentary column. Thus, in a manner analogous to the enumeration of tree rings, the varves in such a column may be counted to date a particular layer. The correlation of rainfall and sedimentation patterns and ²¹⁰Pb radiometric ageing⁵ confirms these dates.

Two different coring techniques were employed to cover different but overlapping time scales. A deliberate box corer, designed by A. S. to obtain sizable cores of soft material without substantial disturbance, was used to obtain samples of recent sediments. Three such cores were collected between 1968 and 1971 within 3 mile of each other in the Santa Barbara Basin (approximately 34° 51' N, 120° 01' W) at a depth of about 580 m. The period covered was 1825 to 1971. A fourth core from the same area, collected in 1966 with a Bootstrap corer⁶, obtained a much deeper sample of the sedimentary column. The upper portion of this core was identifiable as falling in the period 1820-1845, and thus overlapped the bottom layer in the deepest box core. The dating of the bottom layer in this core was less precise, but we estimate it was deposited ~1,500 yr BP.

The box cores were frozen on collection, but the Bootstrap core was sectioned and stored unfrozen. All cores were subsequently split and X-rayed for detailed examination. After correlating unique layers, absolute dates of the varves were established. A total of thirty-eight dated subsamples from the four cores were analysed for mercury content. Sixteen of these, covering a span of 150 yr, were analysed both by neutron activation analysis (NAA) and by flameless atomic absorption spectrometry (FAAS). The average ratio of the results from these two methods (NAA/FAAS) on the sixteen samples was 1.2, and the 95% confidence interval of the mean ratio was 0.9 to

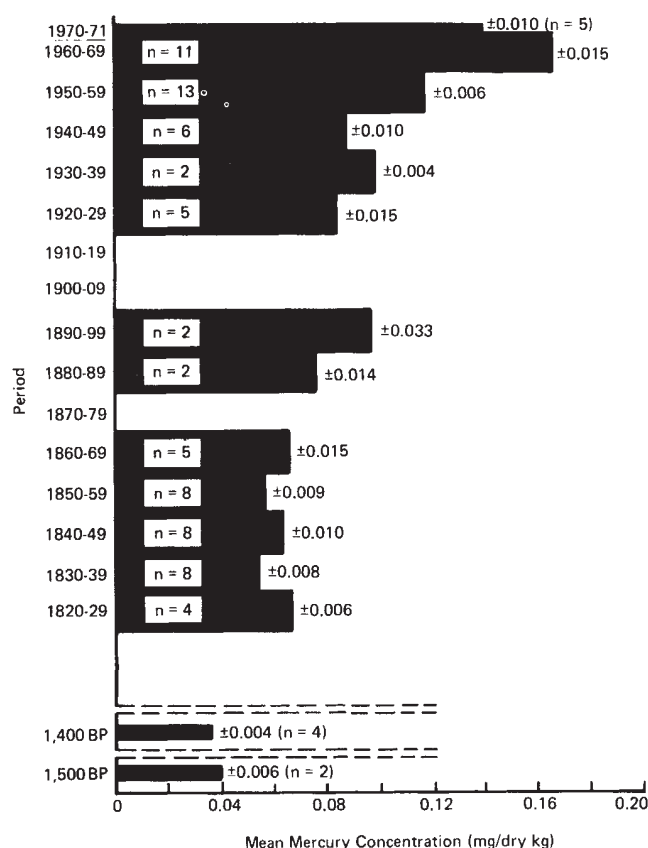


Fig. 1 Mean concentrations of mercury in dated varved sediments from Santa Barbara Basin. Number to right of bar indicates standard error.

1.5. Because the results from the two independent techniques were not significantly different, we consider all the data to be of equal reliability. We note that for each of the decades falling within the periods 1820–1869 and 1920–1971, there are approximately an equal number of analyses by the two methods. The “decade” averages for 1,500 yr BP, 1,400 yr BP, 1880–1889, and 1890–1899, are based on NAA analyses only. In all, concentrations from eighty-five individual analyses were incorporated into the average values for fifteen specified periods. The results (including s_x and n) are presented in Fig. 1.

The concentrations of mercury in the varved sediments of Santa Barbara Basin show a distinct increase toward the surface. The baseline value for the nineteenth century seems to be $\sim 0.06 \pm 0.01$ mg/(dry kg) with the concentrations beginning to exceed this significantly some time around the turn of the century. In addition, the few samples analysed from the Bootstrap core suggest the possibility of a somewhat lower baseline value for the older sediments. The average value for the layers deposited since 1950 exceeds the estimated nineteenth century baseline value by a factor of approximately two.

This observation may be compared to that of Weiss *et al.*⁷ for the concentrations of mercury in dated glacial samples from Greenland. They found a relatively uniform concentration of mercury in layers deposited between 800 BC and 1950, with an approximate doubling in the average value for the past two decades. They attributed this increase to an increased degassing of the Earth's crust, possibly due to the actions of man. Four other investigations into historical concentrations of mercury have also indicated significant increases in recent decades. Thomas⁸ reported that the concentration of mercury in a dated core from Lake Ontario began to increase above background levels about 1900, presumably due to the commencement of local industrial discharges. Barnes and Schell⁹ observed a similar pattern in

three dated cores from Lake Washington. The increases in mercury concentration also began around the turn of the century, and were attributed to a variety of local sources. Aston *et al.*¹⁰ have recently reported that, in a dated core collected from a rural English lake, sediments deposited since 1900 contained approximately twice the average mercury concentration found in sediments laid down during the nineteenth century. They suggested that such mercury profiles in lake sediments are possible indicators of technological growth. Johnels and Westermark¹¹, working in Sweden, analysed feathers from museum specimens of the great crested grebe (*Podiceps cristatus*), whose diet is principally small fish. Again, the mercury levels began to rise about the turn of the century, and the average value for the past three decades was twice the background concentration. The chlorine-alkali industry, which uses mercury electrodes that permit considerable mercury losses to the environment, was introduced in Sweden about 1900.

Thus, it seems that, during approximately the past 100–150 yr, human activities have resulted in a rather similar pattern of increased mercury introduction to several reservoirs in the northern hemisphere. In those waters relatively close to population centres, increases attributed to various anthropogenic sources are detectable in samples deposited beginning about 1900. In contrast, the levels in the more remote regions of the arctic glaciers reflect a detectable increase only in layers laid down during the past two decades, and this has been attributed to a more general increase in degassing of the Earth's crust rather than specific local or regional inputs. The Santa Barbara Basin dated varved sediments show higher concentrations beginning about the turn of the century, suggesting that increased regional inputs of mercury to the southern California coastal waters occurred about the same time as in several other regions of economic growth in the northern hemisphere. According to this interpretation, the anthropogenic input rate of mercury to this coastal marine reservoir seems roughly to have matched the natural input rate in recent years.

An alternative explanation of the varved sediment mercury profile is that mercury is relatively mobile in the interior of anaerobic sediments and is continuously concentrated in the newly deposited and less anaerobic sediment of the surface layer. At present, the chemistry and mobility of mercury in these marine sediments are too poorly understood to exclude this possibility. Nevertheless, it seems that about a factor of two may be placed on the extent to which man recently has increased, on a regional scale, the level of mercury in the southern California marine ecosystem.

The sediment samples were obtained with the help of Eric Duffrin, and the captain and crew of RV E. B. Scripps. Dr J. Galloway, University of California, San Diego, provided initial guidance in the FAAS measurements. R. Simpson and Deirdre McDermott, Southern California Coastal Water Research Project, provided editorial and technical assistance. This research was supported by the Southern California Coastal Water Research Project and the Marine Life Research Program at Scripps Institution of Oceanography, the latter sponsored by the Marine Research Committee of the State of California.

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¹ *New Scientist*, 48, 534 (1970).

- ² Johnels, A. G., Westermarck, T., Berg, W., Persson, P. J., and Sjöstrand, B., *Oikos*, **18**, 323 (1967).
- ³ Seagran, H. L., *Limnos*, **3**, 3 (1970).
- ⁴ Klein, D. H., and Goldberg, E. D., *Environ. Sci. Tech.*, **4**, 765 (1970).
- ⁵ Koide, M., Soutar, A., and Goldberg, E. D., *Earth Planet. Sci. Lett.*, **14**, 442 (1972).
- ⁶ Isaacs, J. D., and Brown, D. M., *J. Sedim. Petrol.*, **38**, 159.
- ⁷ Weiss, H. V., Koide, M., and Goldberg, E. D., *Science*, **174**, 692 (1971).
- ⁸ Thomas, R. L., *Canad. J. Earth Sci.*, **9**, 636 (1972).
- ⁹ Barnes, R. S., and Schell, W. R., in *Cycling and Control of Metals*, 45 (National Environmental Research Center, Cincinnati, Ohio, 1973).
- ¹⁰ Aston, S. R., Bruty, D., Chester, R., and Padgham, R. C., *Nature*, **241**, 450 (1973).
- ¹¹ Johnels, A. G., and Westermarck, T., in *Chemical Fallout* (edit. by Thomas, C. C.), 221 (Springfield, Illinois, 1969).

Age of the Huntsman Limestone (Bulawayan) Stromatolites

THE Huntsman limestone stromatolites were originally described by Macgregor¹. Recent work (refs 2-5 and T. Hoering (personal communication)) has established their organic origin beyond reasonable doubt, and shown that macro, micro and chemofossils occur together in the Huntsman limestone. Far less attention has been paid to the stratigraphic and absolute age relations of the limestone in which they occur. As a result of recent detailed field work in and around the quarries, and a comprehensive attack on the ages of granitic events in the southern half of Rhodesia, we can now give a revised estimate of the age of these ancient organisms.

Cloud² crystallized doubts on the true age of the Huntsman fossils when he wrote "... the pegmatitic rocks which have been used to place this minimum age (2.6 aeons) on the Bulawayan, occur over 500 kilometres from the stromatolitic structures and there is, therefore, at least a little room for reservation about their age. . . ." A further cause for doubt is introduced by the seemingly low grade of metamorphism of the limestone. This suggests the possibility that it is a much younger rock, introduced into the area tectonically.

The regional geology was mapped and described by Macgregor, Ferguson and Amm⁶. The Huntsman limestone was included without question in the lower part of the "Zwankendaba Group" of the Lower Series of the Volcanic Greenstone System, which later became the Bulawayan System (now Group). Lenticular limestone is associated with more persistent Banded Ironstones, and outcrops at intervals for several miles along strike. Limestone has been quarried only at Huntsman, and fossils are not known from the other limestone occurrences.

Very detailed mapping was undertaken by one of us (N. J. W.) around the Huntsman quarries. This has shown that the stromatolitic limestone is interbedded in normal sequence with typical Bulawayan rock types. The succession in descending order is:

Greenstone
(dolerite sill)
Greenstone
Upper limestone
Banded ironstone
Lower limestone (with stromatolites)
Quartz sericite schist

The field relations are shown in Fig. 1.

The tectonic style conforms to the general regional pattern of the Bulawayan rocks in the district. The area is faulted, but there is no sign of the important structures which would be necessary to implant a slice of much younger rock in the area.

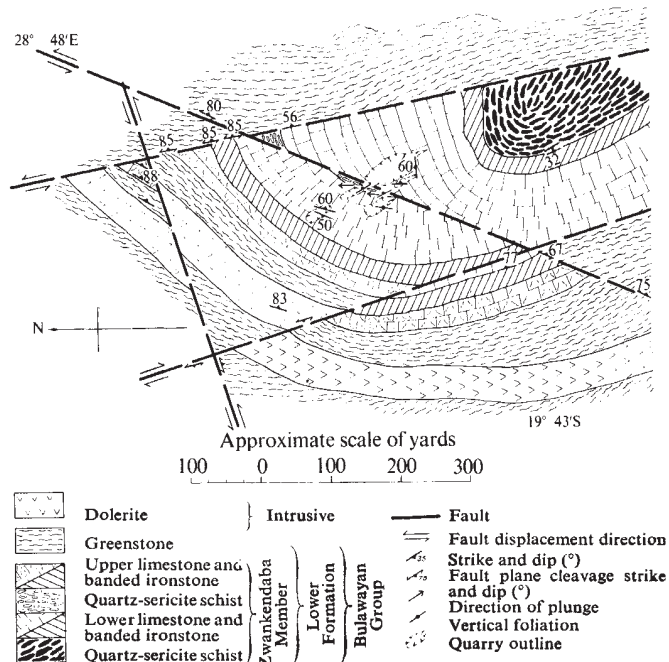


Fig. 1 Field relations, Huntsman quarries.

The metamorphic grade of the limestone could not be determined directly as no impure carbonate rock with calc-silicate minerals could be found. But X-ray diffraction on the insoluble residue shows that some of the carbon is present as graphite; scattered sulphide minerals also occur in the limestone. It can be said, therefore, that structurally, stratigraphically and in metamorphic grade the succession which includes the Huntsman limestone is part of the Bulawayan Group in the type area, and lies low down in the succession.

The age quoted by Cloud depended upon the age of the Bikita pegmatite 500 km to the east, which is intrusive into what appear to be typical Bulawayan greenstones. But lithological correlations of such ancient rocks over such a distance must be treated with caution.

Wilson⁷ obtained three conventional K-Ar age determinations for each of thirty-nine samples from a variety of granitic rocks in the southern part of Rhodesia. Some of these are in the Bulawayo region, and the relevant sample sites, with their ages, are shown in Fig. 2. Two other ages which have become known recently are included on the map. We list the available information in Table 1.

Table 1 Summary of Available Data

Intrusive rock	Age (m.y.)	
Bulawayo porphyritic granite	2,844 ± 85	K-Ar conventional method
Bulawayo porphyritic granite	2,899 ± 87	
Essexvale granite	2,848 ± 57	
Heany granite	2,962 ± 59	
Hillside syenite	2,603 ± 78	
Formona granite	2,670 ± 110	Rb-Sr

There are, therefore, representatives of both Wilson's 2.9×10^9 yr and 2.6×10^9 yr events⁷. Because the outcrop of the Bulawayan Group is continuous throughout the area, and the Heany, Essexvale and Bulawayo granites are intrusive into those rocks, we conclude that the age of the Huntsman limestone and its fossils must exceed 2,900 m.y., and is probably greater than 3,000 m.y. This is considerably older than the 2,600 m.y. minimum age previously assigned to these fossils, and would make them

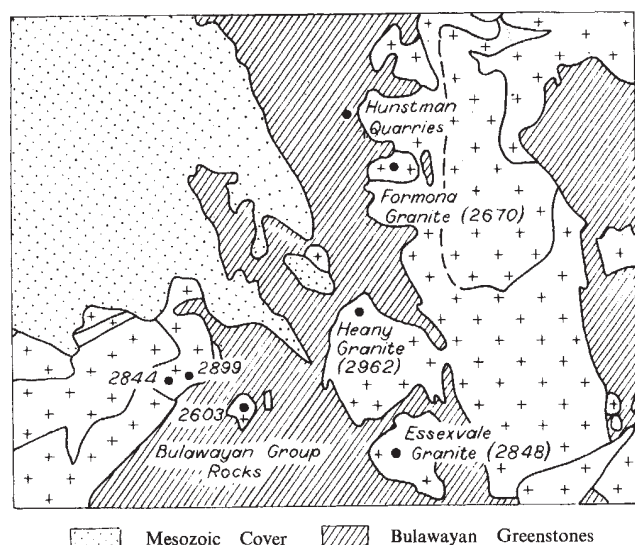


Fig. 2 Distribution of dated rocks in the Bulawayo area. Scale 1 : 720,000.

the oldest occurrence of combined macro, micro and chemo-fossils known.

It also makes the statement by Molyneux⁸ in 1901 that "... some of the ancient Rhodesian schists are of sedimentary origin and are worth searching for microfossils..." sound almost prophetic.

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- ¹ Macgregor, A. M., *Trans. geol. Soc. S. Afr.*, **43**, 9 (1940).
- ² Cloud, P. E., *Science*, N.Y., **148**, 27 (1965).
- ³ Barghoorn, E. S., and Schopf, J. W., *Science*, N.Y., **150**, 337 (1965).
- ⁴ Oberlies, F., and Prashnowsky, A. A., *Z. Naturw.*, **1**, 25 (1968).
- ⁵ Cloud, P. E., and Semikhatov, M. A., *Am. J. Sci.*, **267**, 1017 (1969).
- ⁶ Macgregor, A. M., Ferguson, J. C., and Amm, F. L., *Geol. Surv. S. Rhod. Bull.*, **30** (1937).
- ⁷ Wilson, J. F., *Phil. Trans. R. Soc. A*, **273**, 389 (1973).
- ⁸ Molyneux, A. J. C., *Proc. Trans. Rhod. Sci. Ass.*, **2**, 88 (1901).

Syntectonic Recrystallization and Texture Development in Quartz

I HAVE been carrying out an electron microscopy study of quartz which has undergone partial or complete recrystallization during natural deformation. This study has shown that syntectonic recrystallization and texture (fabric) development can be interpreted by dislocation processes similar to those observed in deforming metals and ceramics rather than by the crystal growth under non-hydrostatic stress proposed in the literature¹⁻³.

The quartz specimens used were from the deformed Arunta quartzite, Central Australia^{4,5}, deformed quartz pegmatites, Ness, Lewis⁶, and Moine mylonites, Eriboll, Scotland⁷. When examined in a petrological microscope, specimens from the first two localities showed the progressive recrystallization of deformed ribbon-like grains (Fig. 1a, b) which had their *c* axes concentrated perpendicular to their direction of elongation. The deformed Arunta quartz grains contained sub-basal deformation lamellae which were gradually replaced by subgrains as the recrystallized areas adjacent to the grain boundaries were approached. Only subgrains were observed within

the deformed Ness quartz grains and they were of a similar size (20 to 50 μ m) to the newly recrystallized grains. The outline of relic grains could be distinguished with the aid of a sensitive tint in partially recrystallized areas. This suggests that the newly formed grains have orientations similar to their parent grains and become more randomly orientated as the strain increases. The mylonite contained small, flattened quartz grains. Again a relic structure consisting of larger "streaked out" grains was detected in some specimens, indicating that these mylonites formed by the recrystallization of an earlier structure.

Foils representing each stage in the progressive recrystallization seen in the Arunta and Ness specimens, and also from areas in the mylonites exhibiting the relic grain structure, were prepared for electron microscopy by ion thinning. All foils were examined in an AEI EM7 electron microscope operating at 1,000 kV. The deformation lamellae in the Arunta quartzite were the walls of long irregular subgrains which were sub-parallel to the basal plane (Fig. 1c). As the recrystallized areas were approached these shortened first into rectangular (Fig. 1e) and then into square (Fig. 1f) box-like subgrains, by the development of prismatic and rhomb dislocation walls. The shortening was accompanied by a progressive rotation of the sub-basal walls into a rhomb position. Thus the final product comprised small equidimensional subgrains bounded by walls approximately parallel to rhomb planes. These subgrains were much smaller than those seen optically and formed a subgrain in subgrain structure⁸. The internal dislocations within the subgrains changed from dense forests of $\langle 11\bar{2}0 \rangle$ (0001) dislocations (Fig. 1d) to a low density, recovered structure consisting of bowed, looped and jogged dislocations (Fig. 1f, g). The former dislocation structure caused the undulatory extinction approximately perpendicular to the deformation lamellae. Small subgrains with recovered internal dislocations also occurred in the recrystallized grains.

The deformed Ness quartz grains had a well developed subgrain in subgrain structure. The large more misorientated subgrains (misorientation normally $>5^\circ$) could be directly related to the optical subgrains. The small subgrains within these had only slight misorientations (1° or less) and could not be seen in the optical microscope. They contained internal recovered dislocation structures. The small recrystallized grains also contained small subgrains with similar internal dislocation structures (Fig. 1h). In the electron microscope the recrystallized areas could only be distinguished from adjacent parent grains by the preferential etching of their grain boundaries (Fig. 1i). The mylonite quartz also contained small subgrains with internal recovered dislocation structures.

So the recrystallized grains which were seen optically were not strain-free nuclei but clusters of subgrains sufficiently misorientated from their neighbours to be visible as individual entities. This clustering was first evident as optical subgrains which apparently became more misorientated and formed the recrystallized grains. This type of recovery and recrystallization process obviously differs from static (annealing) recrystallization which produces dislocation-free (strain-free) nuclei⁹. It is, however, similar to the dynamic recovery and recrystallization which occur in metals and ceramics during steady state deformation¹⁰⁻¹⁴ and in ice during glacier flow¹⁵. But the textures produced by the two processes differ. Static recrystallization results in well defined grain orientations¹² whereas dynamic recrystallization progressively randomizes the texture formed by the intracrystalline slip processes. Syntectonic recrystallization is a dynamic process. Thus, by combining my observations with the relevant metallurgical data¹⁰⁻¹⁴, a sequence which explains the formation of syntectonic quartz textures can be outlined. The stages are the following:

(1) Initial deformation by intracrystalline slip. This results in a preferred orientation (such as *c* axis maximum texture) of the quartz dependent upon the operative slip system or systems and grain elongation parallel to the local tensile axis. Undulatory extinction forms in the grains.

(2) Initiation of dynamic recovery. This is triggered by the

increase in the internal strain energy of the quartz because of the increased dislocation density. Dislocation movement by cross slip, and particularly by thermally activated processes such as climb, commences. Elongated subgrains form sub-parallel and perpendicular to major slip planes. There is no change in grain shape or orientation; however, deformation lamellae and deformation bands (narrow elongated subgrains within larger elongated subgrains⁸) form.

(3) Continued recovery and reduction in the internal dislocation density. The elongated subgrains become equiaxed and cluster within higher angle boundaries to form the larger subgrains which can be detected with an optical microscope.

(4) Further recovery. An increasing number of dislocations enter the higher angle boundaries causing the clusters of subgrains to rotate and appear optically as the small recrystallized grains. Because these grains are now individual and distinguishable entities this stage can be termed "dynamic recrystallization". A fine-grained quartz is formed with a crystallographic texture rather similar to that of the former grains, the outline of which can still be detected with a sensitive tint.

(5) Continued rotation and growth of the new grains by stress-induced grain boundary migration. All evidence of the old texture is destroyed. The new texture will become progressively more random as the strain increases; however, the predominance of rhomb and prism boundaries precludes a completely random breakup of the old texture and new textures such as *c* axes, small circles and girdles are formed.

(6) Decreasing rotation and growth of the now enlarged recrystallized grains. The serrated grain boundaries formed by migration interlock and create internal stresses which increase the rate of dislocation generation. These dislocations in turn equilibrate the internal strains in adjacent grains and boundary migration ceases. The increase in intracrystalline slip which may lead to the elongation of the recrystallized grains brings about a further phase of subgrain formation (recovery) and recrystallization—the process recommences.

This sequence is one of continual dynamic recovery of which dynamic recrystallization is a part during a basically steady state flow. There is no sharp demarcation between each stage; one gradually passes into the other. Recovery features can and do coexist with one another, for example, deformation lamellae with subgrains. Individual grains, and even different parts of a single grain, may be at different stages in this sequence due to the inhomogeneity of the local stresses and strains in a deforming polycrystalline body. The predominance of any one of the above six stages during a deformation will depend upon the temperature, strain rate (and differential stress) and, for quartz, the lattice "water" (silanol) content. Low temperatures and normal strain rates or, more particularly, high temperatures and high strain rates will favour stage (1), and quite large grain elongations will be possible before recrystallization. Recovery processes will dominate during the slow deformation of wet

quartz at moderate and high temperatures. Exaggerated grain growth can modify the crystallographic texture by eliminating those grains that have rotated into an unfavourable orientation. A high strain rate deformation at moderate to high tempera-

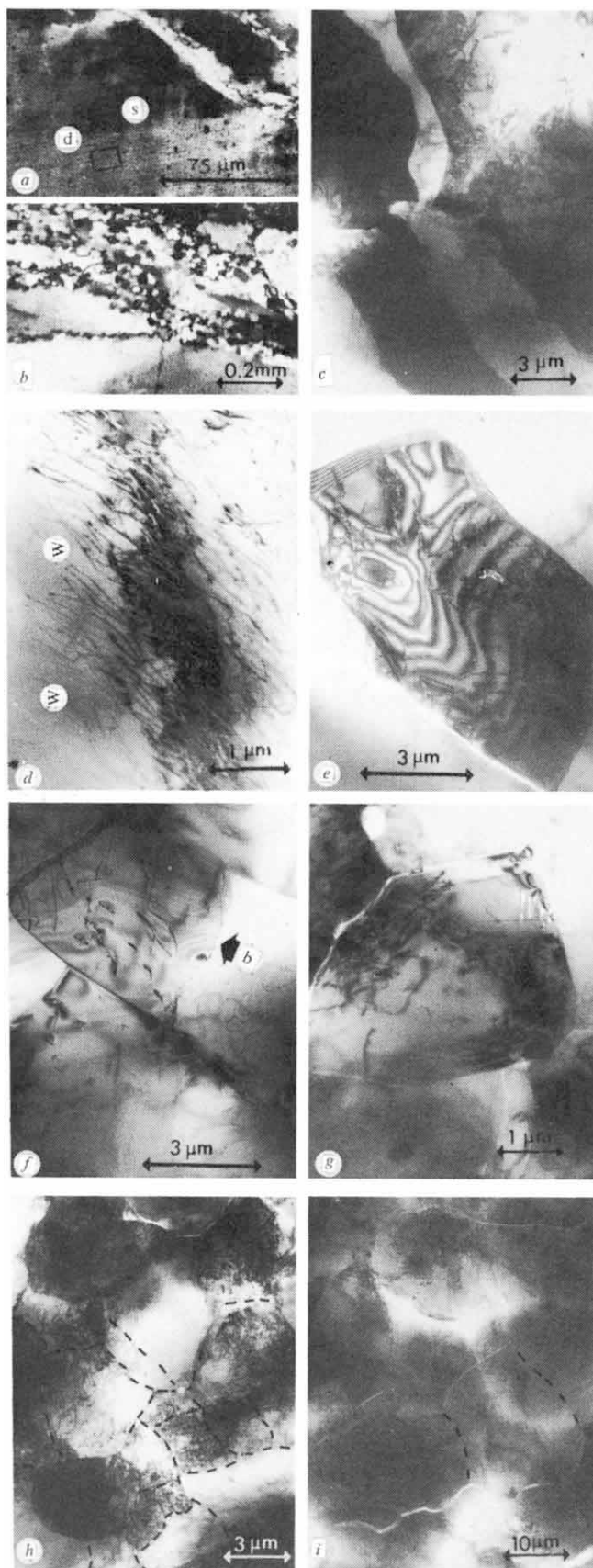


Fig. 1 *a*, Subgrains, *s*, replacing deformation lamellae, *d*, adjacent to recrystallized areas in the Arunta quartzite. The marked area in the lower left corner shows the size of the area covered by the micrograph in *c*. *b*, Recrystallized areas in elongated quartz grains in the Ness quartz. *c*, Long subgrains approximately parallel to the basal plane seen optically as deformation lamellae. The dislocations within the central subgrain are out of contrast. *d*, A dislocation forest in a long subgrain. A subgrain wall is visible along *ww*. *e*, A rectangular subgrain. *f*, The breakup of a rectangular subgrain by an internal low angle boundary, marked *b*. *g*, An equiaxed subgrain adjacent to a recrystallized area. *h*, Equiaxed subgrains in a recrystallized area. Those subgrain walls that are not clearly visible have been marked in. *i*, Etched out high angle boundaries which surround clusters of subgrains. The marked lines are boundaries which did not etch out completely. (*a* and *b* are optical micrographs; *c* to *i* are electron micrographs; *c* to *g* from the Arunta quartzite and *h* and *i* from the Ness quartz.)

tures should result in cyclic deformation and recrystallization accompanied by progressive grain refinement. Small elongated, rather than equidimensional, grains may form under such conditions. It is possible that many quartz mylonites, especially the primary mylonites along the Moine thrust and in particular the blastomylonites, may have formed by this process, with the grain elongation being parallel to the tensile direction. Larger grains (porphyroclasts) in these mylonites are possible areas at a different phase, or in a different stage in the same phase, of dynamic recovery.

Complications will arise if the temperature or strain rate alters during deformation. If the former drops and/or the latter increases, more dislocations will be generated than can be annihilated by the dynamic recovery processes and the internal dislocation density will increase. A new phase of recovery will be initiated and features such as deformation bands and lamellae or a new phase of recrystallization result. If the operative slip system or systems are temperature dependent then a new crystallographic texture forms in stage (1) and will progressively overprint and eventually replace the existing texture. The overall result is that different textures can arise in separate areas of one rock during a single deformation. Also, deformation features such as lamellae and bands can form at different periods during deformation and cannot be related to any specific strain value.

I suggest that texture development in most crystalline metamorphic quartzites should be interpreted in terms of progressive deformation and dynamic recovery, with dynamic recrystallization being an integral part of the recovery sequence. Also, because the crystallographic texture of a quartz tectonite will depend upon such variables as deformation conditions, operative slip systems, the phase recrystallization preserved, degree of grain growth and the stage at which deformation and metamorphism ceased, as well as the preferential formation of subgrain walls with rhomb and prism walls, it seems unlikely that there should be more than a tenuous relationship between the symmetry of a crystallographic texture and the symmetry of the regional stresses.

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- ¹ Voll, G., *Liverpool Manchester geol. J.*, **2**, 503 (1960).
- ² Kamb, W. B., *J. geophys. Res.*, **66**, 259 (1961).
- ³ De Vore, G. W., *J. Geol.*, **77**, 26 (1969).
- ⁴ Watson, J., *Scottish J. Geol.*, **5**, 270 (1969).
- ⁵ Forman, D. J., Milligan, E. M., and McCarthy, W. R., *Bur. Min. Res. Aust.*, Rep. 103 (1970).
- ⁶ Wells, A. T., Forman, D. J., Ransford, L. C., and Cook, P. J., *Bur. Min. Res. Aust. Bull.*, 100 (1970).
- ⁷ Soper, N. J., *Scottish J. Geol.*, **7**, 241 (1971).
- ⁸ White, S. H., *J. Mater. Sci.*, **8**, 490 (1973).
- ⁹ Hu, H., in *Recovery and Recrystallization of Metals* (edit. by Himmel, L.) (Interscience, New York, 1963).
- ¹⁰ Hardwick, D., Sellars, C. M., and Tegart, W. J. McG., *J. Inst. Metals*, **90**, 21 (1961/62).
- ¹¹ Garafalo, F., *Fundamentals of Creep and Creep Rupture in Metals* (Macmillan, New York, 1965).
- ¹² Honeycombe, R. W. K., *The Plastic Deformation of Metals* (Arnold, London, 1968).
- ¹³ *Deformation under Hot Working Conditions* (edit. by Sellars, C. M., and Tegart, W. J. McG.) publication 108 (Iron and Steel Institute, London, 1969).
- ¹⁴ Jonas, J. J., Sellars, C. M., and Tegart, W. J. McG., *Met. Rev.*, **130**, 1 (1969).
- ¹⁵ Paterson, W. S. B., *Physics of Glaciers* (Pergamon, Oxford, 1969).

Biological Method of Estimating Smoke and Sulphur Dioxide Pollution

SMOKE and sulphur dioxide levels are measured by the Warren Spring Laboratory at many sites in Great Britain¹, but little information is collected in rural areas. Here we describe a method for estimating atmospheric pollution which can be applied in most areas where trees are found.

Atmospheric pollution has a deleterious effect on much living matter and this may be cumulative². A study of living matter may therefore give an indication of the pollution levels to which an area has been subjected in the recent past. Although effects are seen in animals as well as in plants, the mobility of the former renders them less suitable as local indicators.

Most attention has been paid to the occurrence and growth forms of lichen and bryophyte species (see, for example, refs 3 and 4). Although there seems to be close agreement between these parameters when used as indicators, we are not aware of any attempt to combine several independent measures into a single quantitative assessment. As part of a study of the effects of air pollution on insects⁵, we have made measurements at 104 sites in southern Britain of several parameters which may be influenced by air pollution, and here we indicate how they may be combined to provide estimates of smoke and sulphur dioxide levels by multiple regression.

The 104 sites were in closed woodland with a herbaceous understorey. At each, ten oak trees (*Quercus robur*, *Q. petraea* and hybrids) more than 20 cm in diameter at 1 m from the ground were sampled. Homology between the sites was further ensured by the exclusion from the sampling of marginal trees and those bordering pools, streams and marshy areas. The parameters measured in 1969 and 1970 were: the number of bryophyte species present on the boles of the trees; the maximum height at which they were found (excluding rain water runoff channels); the proportion of the bark at 1.5 m covered with algae of the genus *Pleurococcus*, crustose and foliose lichens (the remainder being bare bark); and the mean reflectance of the trunk at the same height. We also included as independent variables the National Grid coordinates of each site (east and north), altitude, mean annual rainfall and mean January and July temperatures⁶.

We prepared contour maps for both smoke and sulphur dioxide by extrapolation and interpolation, using the mean annual values published by the Warren Spring Laboratory¹. To even out the distance between the contours the measure of pollution that we have adopted is the square root of the values (in $\mu\text{g m}^{-3}$). We see no reason to suppose that the estimates consistently tend to be either too high or too low in either urban or rural areas; the errors will in effect be random and we assume that the only influence on the regressions is to reduce their significance.

With the exception of *Pleurococcus* cover, all the parameters relating to oak trees have high linear correlation coefficients with sulphur dioxide and with smoke (Table 1). The two sets of coefficients are similar because smoke and sulphur dioxide are closely correlated ($r=0.88$). The low values relating to *Pleurococcus* are not unexpected because these algae only occur commonly at intermediate pollution levels.

The multiple regression calculations were performed on a computer using University of California program BMD 02R. The variables which, taken successively, add more than 1% to the value of R^2 (the proportion of the total variance accounted for by the regression) are incorporated into the regression equations in Table 2. In each equation $R^2=0.71$. The constants should be recalculated if the method is used in other situations. Rainfall seems to have little influence on the bryophyte parameters compared with that of pollution; precipitation rates at our sites range from 22 inch yr^{-1} to 90 inch yr^{-1} .

The index y_{SO_2} (Fig. 1) reflects the high levels of sulphur

Table 1 Values of the Correlation Coefficient r between the Biological Variables and the Local Pollution Levels at 104 Sites in Southern Britain

	SO ₂	Smoke
Species per tree	-0.80	-0.77
Bryophyte height	-0.75	-0.69
<i>Pleurococcus</i> cover	0.12	-0.02
Crustose lichen cover	-0.63	-0.66
Foliose lichen cover	-0.47	-0.39
Bare bark cover	0.72	0.79
Log _e reflectance	-0.76	-0.75

Units of measurement used: smoke and sulphur dioxide ($\mu\text{g m}^{-3}$)^{1/2}; square root of the mean number of bryophyte species on each of ten oak trees; square root of the mean maximum height at which bryophytes were found on each of the ten trees; foliose and crustose lichens, *Pleurococcus* and bare bark expressed as mean percentile cover at 1.5 m height on the ten trees; mean log_e reflectance of these trees at 1.5 m.

Table 2 Multiple Regression Equations used as an Index of Sulphur Dioxide and Smoke Levels in Figs 1 and 2

- (a) Sulphur dioxide
 $y\sqrt{so_2} = 17.33 - 1.18 \times \text{spp/tree} - 3.09 \times \text{reflectance} - 2.78 \times \text{foliose}$
- (b) Smoke
 $y\sqrt{sm} = 9.71 + 4.55 \times \text{bare bark} - 0.82 \times \text{spp/tree} - 2.14 \times \text{reflectance} + 2.33 \times \text{crustose}$

For units of measurement see caption to Table 1.

dioxide occurring in London (Hampstead Heath, J), Birmingham (Edgbaston, E) and the industrial region of Lancashire (Mytholmroyd, A, and Sefton Park, B) and Yorkshire (Tinsley, C). Locally high values, relative to the surroundings, are found in other urban areas, such as Norwich (N), Bristol (Hanham, H) and Bangor (D). A more extensive area where appreciable pollution levels are found is centred on the valleys of South Wales (Pont Rhyd y Fen, F, and Craig Gwent, G). High values of the index found in Ampthill (K), Sandy (L), and Whittlesey (M) are associated with extensive brick making in an otherwise rural area; here fluoride may add to the effects of sulphur dioxide. Because of the absence of National Survey data, we included values not exceeding $30 \mu\text{g m}^{-3}$ for

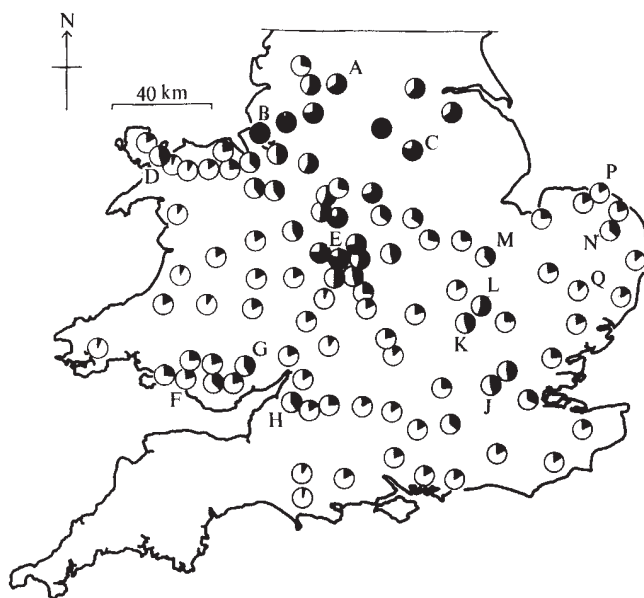


Fig. 2 Map of southern Britain showing values of the index of mean annual smoke levels at 104 sites, derived from equation (b) in Table 2. The black segments are linearly proportional to the amount of pollutant within the range 0 to $100 \mu\text{g m}^{-3}$. Values greater than this maximum are not distinguished.

large rural areas. The index agrees with these lower values for southern England and in Wales. But in East Anglia (such as Upper Sherringham, P, and Woolpit, Q) consistently higher values of y_{so_2} are obtained.

Calculated values of y_{sm} for smoke are shown in Fig. 2. The values reflect the high levels of smoke found in the Lancashire/Yorkshire region (A, B and C) and the progressive decrease in the Birmingham area (E) and in London (J). Locally high y_{sm} values are also obtained in other areas (D, F, H, K, L, M and N) providing a qualitatively, though not quantitatively, similar situation to that for y_{so_2} . By contrast with the relatively high values obtained for y_{so_2} in East Anglia, y_{sm} is generally low in all extensive rural areas.

The mean number of bryophyte species per tree is closely correlated with the more easily measured mean maximum height at which bryophytes are found on the trees ($r=0.89$); the replacement of number of species by height gives the equations appearing in Table 3 for sulphur dioxide ($R^2=0.69$) and for smoke ($R^2=0.70$).

Table 3 Multiple Regression Equations modified from Those in Table 2 by the Substitution of Mean Maximum Height of the Highest Bryophyte in the Place of Mean Number of Bryophyte Species on Each of Ten Oak Trees

- (a) Sulphur dioxide
 $y\sqrt{so_2} = 18.72 - 3.94 \times \text{height} - 0.15 \times \text{height} - 2.38 \times \text{foliose}$
- (b) Smoke
 $y\sqrt{sm} = 9.44 + 5.29 \times \text{bare bark} - 0.09 \times \text{height} - 2.48 \times \text{reflectance} + 2.32 \times \text{crustose}$

For units of measurement see caption to Table 1.

The only survey of Britain comparable with our own is that of Hawksworth and Rose⁷. Although they do not indicate the number of sites surveyed, the detail of their map (Fig. 1) shows a far more intensive coverage than this survey. Quantitative comparison is not possible because they have used mean winter pollution levels whereas we have used mean annual values in calibrating the results. Both surveys show the marked local effects of urban areas. But although Hawksworth and Rose indicate many local effects of air pollution that we have been unable to detect, because of the relatively wide spacing of our sites, their method gives no indication of the apparently high pollutant levels detected by us in the

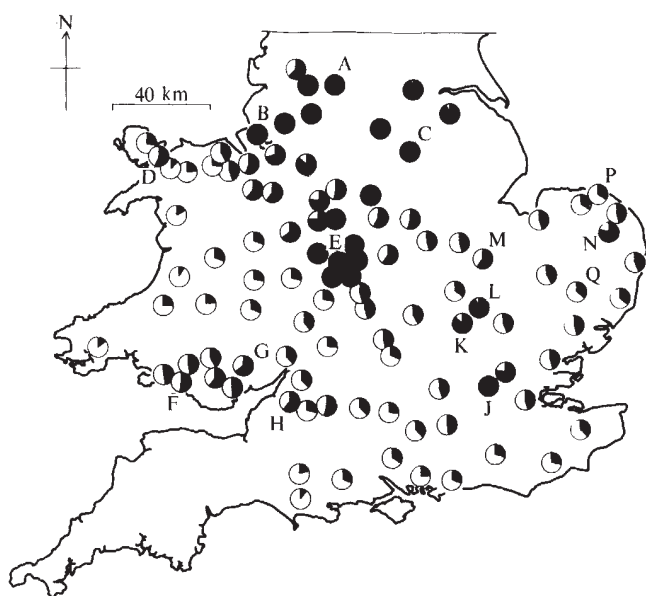


Fig. 1 Map of southern Britain showing values of the index of mean annual sulphur dioxide levels at 104 sites, derived from equation (a) in Table 2. The black segments are linearly proportional to the amount of pollutant within the range 0 to $100 \mu\text{g m}^{-3}$. Values greater than this maximum are not distinguished. Sites indicated by letters are identified in the text.

Reduction of Lens Reflexion by the "Moth Eye" Principle

THE problem of surface reflexion from lenses has led¹ to the development of multilayer interference structures which can suppress the reflexion from glass surfaces by a factor of 10 or more throughout the visible spectrum. But observations on the corneas of nocturnal insects indicate that nature may have anticipated the problems². Electron microscope studies of the corneal lenses of moths reveal that the outer surface is covered in a regular array of conical protuberances, typically of about 200 nm height and spacing. Bernhard² proposed that the function of this structure might be to suppress reflexions by effectively providing a graded transition of refractive index between the air and the cornea. The proposal was substantiated by measurements with microwave radiation reflected from a model of the array, scaled up appropriately for the longer wavelengths.

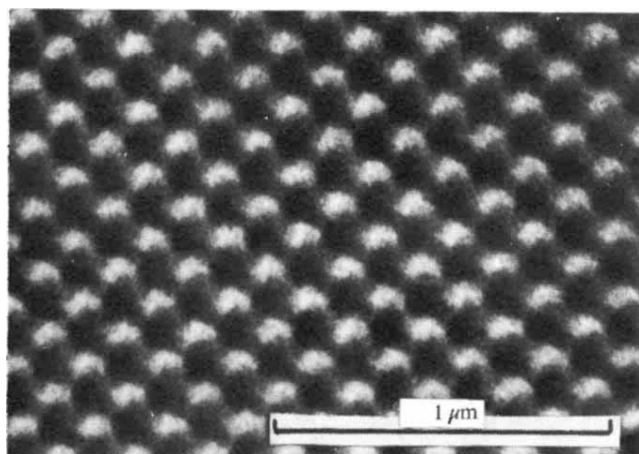


Fig. 1 'Stereoscan' picture of a photofabricated array.

We have prepared a similar array, with dimensions suited to visible wavelengths and have made measurements which confirm the anti-reflexion properties of such a structure. The array was formed in a layer of photoresist coated on a glass substrate, by recording the straight line interference fringes formed at the intersection of two collimated beams of light³. In this case the beams were derived from a krypton laser (wavelength 351 nm) and the included angle was about 120°, giving a fringe spacing of approximately 210 nm. The sinusoidal variation of intensity across the fringes was converted, after suitable exposure and development, into a sinusoidal corrugation in the surface of the resist. By superimposing two such patterns at right angles it was possible to produce a surface structure somewhat similar to that described by Bernhard (Fig. 1).

The results of measurements of specular reflectance made at various wavelength are shown in Fig. 2. In making these measurements, the reflexion from the rear surface of the plate was eliminated by blackening, and the reflexion from the photoresist/glass interface was sufficiently small to be neglected. (The glass was chosen to have a refractive index close to that of the developed photoresist, that is about 1.6.) The integrated "white light" reflectance (conditions of photopic visual response and CIE D6500 standard illumination) at normal incidence was reduced from 5.5% to 0.2%. The transmittance of the same plate with the blackening removed showed an increase of 2.5%. This improvement does not match the reduction in reflectance because of absorption losses in the resist. There was no appreciable scattering from the surface although a weak violet diffracted order was visible in the glass. The reflectance from the

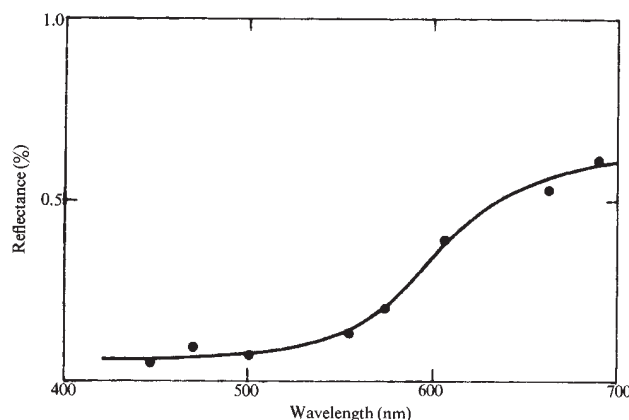


Fig. 2 Measured specular reflectance (normal incidence).

treated surface can, in principle, fall to zero for all visible wavelengths if the geometry of the structure is optimized. The dependence of the reflectance on the effective thickness (d) of the layer of graded refractive index and on the wavelength (λ) of the light was investigated by Rayleigh⁴, and we have computed similar results by representing the graded interface by many layers of equal thickness and progressively increasing refractive index (Fig. 3). When $d \ll \lambda$ the interface appears relatively sharp, and the reflectance is essentially that of a discontinuous boundary. As d/λ increases, however, the reflectance falls monotonically to a minimum value at $d/\lambda \approx 0.4$. Further increases in d/λ show a series of successive maxima and minima in reflectance, but nowhere does the value approach that of the sharp interface. When $d \ll \lambda$, the reflectance is essentially zero. Thus for optimum performance in the visible region of the spectrum, the thickness of the gradient (the height of the protuberances) should be 250 nm or more. In our case, the significant reflectance and its increasing value for longer wavelengths suggests a thickness rather less than this was obtained. The spacing of the protuberances must clearly be sufficiently fine to avoid losses by diffraction, that is, it must be less than the shortest wavelength involved divided by the refractive index of the material, in practice about 250 nm.

In its optical performance the dimpled surface offers an attractive alternative to multilayer antireflexion coatings. The suppression of reflexion over a wide spectral range is comparable with the best multilayer coatings commercially available. Although we have demonstrated that it is possible to produce such a surface, our experiments have been restricted to plane surfaces, and the resulting "coatings" have been extremely delicate. Clearly, considerable techno-

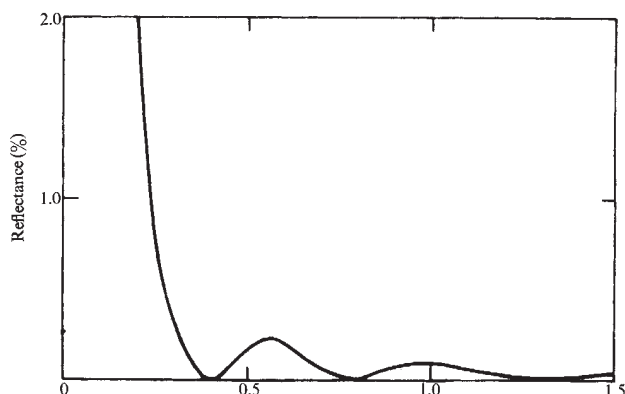


Fig. 3 Computed dependence of reflectance on d/λ .

logical development would be required before the process could become a practicable alternative to multilayer coating.

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¹ Cox, J. T., and Hass, G., in *Physics of Thin Films* (edit. by Hass, G., and Thun, R. E.) 2, 239 (Academic Press, New York, 1964).

² Bernhard, C. G., *Endeavour*, 26, 79 (1967).

³ Auton, J. P., and Hutley, M. C., *Infrared Phys.*, 12, 95 (1972).

⁴ Lord Rayleigh, *Lond. Math. Soc.*, 11, 51 (1880).

Ion Microprobe Confirmation of Pb Isotope Ratios and Search for Isomer Precursors in Polonium Radiohaloes

RADIOHALOES associated with decay of several Po α emitters^{1,2} have been studied by optical microscopic techniques and more recently by mass spectrometric examination of the halo inclusion using ion microprobe techniques^{3,4}. In such cases a large excess of ²⁰⁶Pb compared with ²⁰⁷Pb was found to be incompatible with the radiogenic decay of ²³⁸U and ²³⁵U, yet was explainable on the basis of polonium decay independent of uranium³. A straightforward attempt to account for the origin of these Po haloes by assuming that Po was incorporated into the halo inclusion at the time of host mineral crystallization meets with severe geological problems: the half-lives of the polonium isotopes ($t_{1/2} = 3$ min for ²¹⁸Po) are too short to permit anything but a rapid mineral crystallization, contrary to accepted theories of magmatic cooling rates.

This dilemma might be resolved (R. V. G., unpublished) if several long half-life high-spin or shape isomers of polonium (or the β -decaying precursors) were formed at nucleosynthesis and were subsequently incorporated into the halo inclusions during crystallization. This hypothesis eliminates the geological difficulties, and is open to experimental verification using several techniques such as charged particle reactions, though the long half-lives may present an obstacle. But long half-lives imply that some of the isomers may still exist, in which case a mass analysis of the polonium halo inclusions should reveal whether significant quantities are still present. We now report additional ion microprobe analyses of these Po inclusions as well as U inclusions in search of the isomers and for additional information on the Pb isotope ratios.

Mass scans were taken on areas of the biotite free from haloes. All the normal elemental constituents as well as some trace elements were seen in these scans. The mass region from 150 to 300 is conspicuously free from any mass peaks. Generally Fe_2^+ at position 112 is the only high mass peak of significance observed from the biotite itself.

In the pure uranium, thorium, or uranium-thorium inclusions, ion microprobe analysis showed that the inclusions were either zircons or monazites; in many cases the ²⁰⁶Pb ion current or signal was near background, so that it was difficult to make a common Pb correction; the ²³⁸U/²³⁵U ratio was normal in inclusions which contained uranium; and the ²³⁸U/²⁰⁶Pb signal ratio varied from 10 to 70 in the different inclusions analysed. The actual ²³⁸U/²⁰⁶Pb atom ratio is difficult to determine because of the uncertainty in the U and Pb secondary ion yield from different minerals. In general, U is detected with several times greater efficiency than Pb. The radiogenic ²⁰⁶Pb/²⁰⁷Pb ratio was

difficult to evaluate in those inclusions where the ²⁰⁴Pb signal was near background. In other cases it was found to vary within normal limits.

There is a wide spectrum in the U and Th halo types—some inclusions contain just U or Th without the other element, while other inclusions contain varying amounts of U and Th and in some cases exhibit rings from both decay series; it seems that the same situation prevails with Po and U type haloes in certain micas. In the analyses thus far it seems that the larger the Po halo inclusion the greater the U content tends to be; but more work is needed to verify this. Also the larger inclusions seem to be definite mineral types (usually rare earths but not specifically identified as yet), whereas some of the point-like Po halo inclusions consist of only elemental Pb (without ²⁰⁴Pb) and Bi. Previously no detectable U was found in such cases as the latter type.

In contrast to the Pb ratios in the U and Th halo inclusions, we again report exceptionally high ²⁰⁶Pb/²⁰⁷Pb ratios which are characteristic of the ²¹⁸Po decay sequence type Po halo. The results may be summarized as follows: ²⁰⁶Pb/²⁰⁷Pb ratios of 10, 12, 18, 22, 25, 40, and 100 were observed. In four of these cases no ²⁰⁴Pb was detected. In the other two cases ²⁰⁴Pb was almost background, so that no common Pb correction was made on any of the ratios (any such correction would have produced a larger ²⁰⁶Pb/²⁰⁷Pb ratio). In three of the cases (10, 12, and 22) the small uranium signal seen was 10 to 100 times less than that required to support the Pb observed. These results confirm the earlier ion microprobe analyses of Po halo inclusions in which Pb ratios were found that were impossible to explain on the basis of U decay. They give confidence that we are indeed dealing with a class of haloes that is distinct from the ordinary U and Th types as the optical microscopic measurements invariably suggest. Otherwise, the most important aspect of the results is that the decay product of the polonium (Pb) still exists in these inclusions in measurable quantities (10^8 – 10^{10} atoms) and has not diffused away. On such a basis we then expect that any isomer precursor of Po, if the half-lives were sufficiently long, would also still exist and be detectable by ion microprobe techniques.

The only source of geochemical data about the postulated isomer is derived by inference from the type of halo inclusion. Some Po halo inclusions are of the rare earth variety while others contain only elemental Pb and Bi. The latter case might suggest the existence of an isomer geochemically similar to those elements, whereas the former case is rather non-specific. Fortunately ion probe mass analysis techniques do not depend on knowing the chemical identity of the postulated isomer.

To obtain these Pb ratios, we first cleaved the mica until the halo inclusion appeared on the surface. In some cases the sample was coated with a thin conducting layer of carbon, but it was better to overlay the sample with electron microscope-type Cu grids. In the latter case there was no extraneous material introduced anywhere near the region of interest. Before taking mass scans on the Po haloes the ion microprobe was optimized to obtain the best Pb signal from large U type halo inclusions that were mounted on the same sample but in a different area. In many cases the ion probe was peaked on mass 206 position and then moved to the area in the vicinity of the Po halo inclusion. The signal at this mass position remained at background (1 Hz) until the beam was shifted to the Po inclusion itself. In some cases several minutes elapsed before the signal reached maximum intensity. Generally mass positions 204, 207, 208, 218 and 238 were monitored, as well as the regions considerably below Pb, for possible interference from molecular ions. In other cases mass scans of the entire region from mass 1 to 250 were taken. It can be definitely stated that the exceptionally high 206 signal, compared with 207, occurs only in the Po halo inclusions and is not an artefact due to a molecular ion originating with the mica itself, the inclusion, or a combination of the mica and the elemental constituents of the inclusion. This is not to say the ion microprobe does not generate molecular ions, for in certain cases it does so very efficiently. But in

the case of the Po haloes, we took care to monitor the various possibilities, which could have interfered with the results.

The search for the isomer consisted of carefully scanning the region around mass 218, for the Po haloes used in these experiments originated with ^{218}Po α decay. To be certain of the mass position, a small amount of Hg was placed on the sample holder to use as a mass marker at the 218 position ($^{202}\text{Hg}^{16}\text{O}$). In all Po inclusions except one no signal was observed at the 218 position. That one exception was due to interfering HgO ions from the presence of Hg in the inclusion itself.

A very rough estimate of what these results mean in terms of the present existence of the isomer in the inclusion may be obtained because the ^{206}Pb sputtered ion count rate was greater than 1,000 Hz in some Po inclusions. If it is assumed the isomer resembles Pb in sputtered ion efficiency (Pb has a relatively poor sputtered ion yield), then the present abundance of the isomer in the inclusion is $\leq 10^{-3}$ that of the ^{206}Pb . One interpretation of these results is the isomer has simply decayed to the point where it was not detected in these experiments. (These samples were from an early Precambrian pegmatite in Scandinavia.) It is yet to be determined whether this information is consistent with the half-lives of the proposed isomers that can be ascertained by determining the latest geological epoch in which such haloes occur.

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¹ Henderson, G. H., *Proc. R. Soc., A*, **173**, 250 (1939).

² Gentry, R. V., *Science, N.Y.*, **160**, 1228 (1968).

³ Gentry, R. V., *Science, N.Y.*, **173**, 727 (1971).

⁴ Andersen, C. A., and Hinthorne, J. R., *Science, N.Y.*, **175**, 853 (1972).

BIOLOGICAL SCIENCES

Hypermethylated tRNA in Cleaving Rabbit Embryos

THERE is evidence of a significant transition in the growth characteristics of the mammalian embryo shortly after the onset of blastocyst formation¹⁻³. In those mammals displaying "delayed implantation", extended periods of reversible growth arrest can occur at this time⁴. In the rat, this arrest is clearly a function of embryonic development: cleavage is uninhibited by the hormonally deprived uterus, and arrest occurs only with blastocyst formation⁵. The rabbit embryo, which does not display reversible arrest, nonetheless develops to the early blastocyst stage under various abnormal conditions^{6,7}, whereas further progress to implantation requires, at least transiently, a more complex environment⁸. The molecular basis of this transition in embryonic behaviour in mammals is, therefore, relevant to the problem of the control of cell division. Developmental transitions in a number of biological systems have been

found to be accompanied by alterations in the tRNA methyltransferases and in populations of tRNA⁹, and as tRNA synthesis begins shortly after fertilization in mammalian embryos^{10,11}, we have examined the methylation of tRNA during the preimplantation development of rabbit embryos as an approach to understanding the transition in embryonic growth characteristics. Our findings provide evidence for a marked decrease in tRNA methylation with blastocyst formation.

Preimplantation embryos were exposed to radioisotopes in F-10 solution¹² modified by the inclusion of 10^{-2} M HEPES buffer (Calbiochem) and 5 mg ml⁻¹ dialysed, lyophilized rabbit serum. Embryonic age was estimated by assuming egg fertilization to have occurred 12 h *post coitum*¹³. During labelling, embryos were maintained at 38° C in an atmosphere of 5% carbon dioxide in air at saturation humidity. After extensive washing in saline and homogenization with rabbit liver as carrier, the homogenate was centrifuged at 20,000g for 15 min, and RNA was extracted from the supernatant by the method of Oda and Joklik¹⁴ as modified by Kabat¹⁵. Electrophoretic separation of RNA on 2.5% polyacrylamide gels was carried out as previously described¹⁶. Precursor uptake into the acid-soluble pool was determined by rapidly washing labelled embryos, freezing and thawing them six times in distilled water, and measuring radioactivity in an aliquot of the supernatant following overnight precipitation in 0.3 N perchloric acid and centrifugation at 20,000g for 20 min. Larger blastocysts were removed from the zona pellucida and washed free of blastocoelic fluid before freezing.

The simultaneous labelling of embryonic tRNA with ^{32}P -phosphoric acid and L-methionine (^3H -methyl) facilitates a comparison between the rate of synthesis of the nucleic acid polymer and the rate and extent of its subsequent methylation in the intact embryo. ^{32}P -labelling of tRNA represents true synthesis at all early stages of rabbit embryogenesis, rather than turnover at the -CCA terminus. It is inhibited by actinomycin D¹⁰ and is resistant to *Crotalus* venom phosphodiesterase. The kinetics of uptake of L-methionine and phosphoric acid were similar in cleaving embryos and early blastocysts; pools were saturated by the second hour. Possible variations in precursor pool size at different stages of embryonic development were taken into account by determining the $^3\text{H}/^{32}\text{P}$ ratio in the acid-soluble fraction. The $^3\text{H}/^{32}\text{P}$ ratio in the counts recovered from the 4S region of polyacrylamide gels, corrected by the $^3\text{H}/^{32}\text{P}$ ratio in the acid-soluble fraction, indicates the extent of methylation of newly-synthesized embryonic tRNA. The tRNA methylation decreases approximately six-fold between the fifty-second and eighty-fourth hours of embryonic development (Table 1), a period which coincides with blastocyst formation.

As blastocysts display greater amino acid uptake and incorporation *in vitro* than cleaving embryos¹, the decreased methylation of tRNA in blastocysts is not due to a loss of viability under culture conditions. The ^3H radioactivity in tRNA represents methylation rather than labelling of the purine or pyrimidine rings. 5S RNA, which is not known to be methylated in other cells, had no ^3H radioactivity in these experiments.

Embryonic tRNA labelled with L-methionine (^3H -methyl) alone was analysed for various methylated constituents (Table 2). In 72-h embryonic tRNA, 1- and 7-methylguanine comprised about 1% of the total methylated bases. After 12 h of development, a twelve-fold increase in the proportion of these constituents was found. Alterations in other methylated bases were also observed. The earliest embryonic stage from which sufficient ^3H radioactivity could be recovered for base analysis was the early blastocyst at 72 h following fertilization. It would be of interest to examine the methylated bases at earlier stages. The low levels of total incorporated radioactivity in cleaving embryos, however, would necessitate using very large numbers of animals to acquire that information.

The rate of tRNA synthesis and its methylation, as well as the analyses of methylated constituents in the tRNA, relate to

Table 1 *In vivo* Labelling of tRNA during Pre-implantation Rabbit Development

	Embryonic age (h following egg fertilization)				
	52 (late cleavage)	60	84 (early blastocyst)	108	132 (late blastocyst)
Embryonic cell No. (approximate)	64	128	1,024	9,000	80,000
Total No. of embryos used	63	51	59	36	18
³ H/ ³² P c.p.m. incorporated into tRNA	22.5	14.5	3.7	3.6	2.2
³ H/ ³² P c.p.m. in acid-soluble fraction					

Embryos were exposed to carrier-free ³²P-phosphoric acid (500 μ Ci ml⁻¹, New England Nuclear Corp.) and L-methionine (³H-methyl) (200 μ Ci ml⁻¹, 3.3 Ci mmol⁻¹, Schwarz/Mann) simultaneously in phosphate-free and methionine-free F-10 solution¹² for 4 h. RNA was extracted as described and separated electrophoretically on 2.5% polyacrylamide gels. 4S RNA was excised from these gels and ³H/³²P radioactivity determined by the method of Tishler and Epstein²¹. The ³H/³²P ratio in the acid-soluble fraction was determined at the mid-point of the labelling period, when precursor pools were found to be saturated. The kinetics of uptake of methionine-³H and ³²P were similar in cleaving embryos and early blastocysts. Each value is the average of two determinations.

Table 2 Analysis of Methylated Constituents in tRNA of the Pre-implantation Rabbit Embryo

	Embryonic age (h following egg fertilization)			
	72 (early blastocyst)	84	108	132 (late blastocyst)
Total No. of embryos used	130	62	60	28
³ H c.p.m. in tRNA	4,500	7,000	9,500	12,300
Methylated constituents:				
Methylated cytidylic acids	24	26.7	25.2	10
Methylated uridylic acids	14.5	29.7	15.6	16.2
1- and 7-methylguanine	1	12.4	19.9	13.1
N ² -methylguanine	21	15	15.3	26.3
N ² -dimethylguanine	16	5.4	9.3	11.5
1-Methyladenine	22.1	9.3	12	21.2
6-Methyladenine	0	1.4	2.8	1.5

Embryos were exposed to L-methionine (methyl-³H) (400 μ Ci ml⁻¹, Schwarz/Mann) in methionine-free F-10 solution¹² for 6 h. Cytoplasmic RNA was extracted as described, purified on a DEAE cellulose column, treated with pancreatic DNase (RNase-free, Worthington) and pronase (Calbiochem), deacylated and isolated by gel filtration on 'Sephadex' G100. Acid hydrolysis and thin-layer separation of methylated constituents were carried out by the method of Bjork and Svensson²². The values have been expressed as percentage of radioactivity recovered, and are the average of two determinations. 1-Methylguanine and 7-methylguanine were not adequately separated in most of the analyses, and these values are given collectively.

total tRNA isolated from the entire embryo. Differences in methylation patterns between differentiating embryoblast and trophoblast cells would not be detected, nor would subtle differences in the methylation of individual tRNA species. During preliminary experiments, we found that the relative proportions of individual methylated bases fluctuated in response to differing conditions of *in vitro* labelling. The data reported here, using standardized *in vitro* conditions which are compatible with extended periods of embryonic survival, reflect changes in embryonic tRNA methylation primarily as a function of embryonic age. tRNA synthesis¹⁰ and alterations in tRNA methylation have also been observed very early during sea urchin embryogenesis¹⁷.

The hypermethylation of tRNA in the cleaving rabbit embryo resembles that demonstrated in "transformed" and neoplastic cells⁹. The transition in embryonic growth characteristics with blastocyst formation is accompanied by a marked decrease in tRNA methylation. Methylated bases of tRNA are known to

be determinants in amino acid acceptance¹⁸, codon response¹⁹ and ribosomal binding²⁰. Thus, decreased methylation in rabbit embryos may be directly and causally related to the acquisition of heightened responsiveness to regulatory influences in the reproductive tract.

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- ¹ Manes, C., and Daniel, J. C., *Exp. Cell Res.*, **55**, 261 (1969).
- ² Manes, C., *J. Exp. Zool.*, **172**, 303 (1969).
- ³ Mintz, B., *J. Exp. Zool.*, **157**, 85 (1964).
- ⁴ Enders, A. C. (ed.), *Delayed Implantation* (University of Chicago Press, Chicago, 1963).
- ⁵ Psychoyos, A., *Adv. Biosci.*, **6**, 119 (1971).
- ⁶ Adams, C. E., *J. Endocrinol.*, **16**, 283 (1958).
- ⁷ Brinster, R. L., *J. Reprod. Fert.*, **21**, 17 (1970).
- ⁸ Krishnan, R. S., and Daniel, J. C., *Science*, **158**, 490 (1967).
- ⁹ Borek, E., *Cancer Res.*, **31**, 596 (1971).
- ¹⁰ Manes, C., *J. Exp. Zool.*, **176**, 87 (1971).
- ¹¹ Woodland, H. R., and Graham, C. F., *Nature*, **221**, 327 (1969).
- ¹² Ham, R. G., *Exp. Cell Res.*, **29**, 515 (1963).
- ¹³ Austin, C. R., and Braden, A. W. H., *Austral. J. Biol. Sci.*, **7**, 179 (1954).
- ¹⁴ Oda, K., and Joklik, W. K., *J. Mol. Biol.*, **27**, 395 (1964).
- ¹⁵ Kabat, D., *Anal. Biochem.*, **39**, 228 (1971).
- ¹⁶ O'Melia, A., and Villce, C. A., *Nature New Biology*, **239**, 51 (1972).
- ¹⁷ Sharma, O. K., Loeb, L. A., and Borek, E., *Biochim. Biophys. Acta*, **240**, 558 (1971).
- ¹⁸ Shugart, L., Novelli, G. D., and Stulberg, N. P., *Biochim. Biophys. Acta*, **157**, 83 (1968).
- ¹⁹ Capra, J. D., and Peterkofsky, A., *J. Mol. Biol.*, **33**, 591 (1968).
- ²⁰ Geftter, M. L., and Russell, R. L., *J. Mol. Biol.*, **39**, 145 (1969).
- ²¹ Tishler, P. V., and Epstein, C. J., *Anal. Biochem.*, **22**, 89 (1968).
- ²² Bjork, G. R., and Svensson, I., *Biochim. Biophys. Acta*, **138**, 430 (1967).

Increase in Histamine Receptors on Thymus-derived Effector Lymphocytes during the Primary Immune Response to Alloantigens

THE cytotoxicity of alloantigen-bearing target cells by thymus-derived (T) lymphocytes from appropriately immunized animals has particular relevance to allograft rejection (see ref. 1 for review). Although the mechanism of lysis remains imperfectly understood, recent studies suggest that it consists of multiple steps²⁻⁴ triggered by antigen-lymphocyte interaction⁵, which terminate in the secretion of a cytotoxic mediator protein into or onto the membrane of the target cell^{6,7}.

Drugs which stimulate lymphocyte adenylate cyclase, and thereby increase the intracellular level of cyclic 3',5'-adenosine monophosphate (cyclic AMP), inhibit cytotoxicity⁸⁻¹⁰. This process is reversible; inhibition of cytotoxicity lasts only as long as lymphocyte cyclic AMP levels are elevated. Among the drugs shown to raise lymphocyte cyclic AMP and inhibit T cell-mediated cytotoxicity are prostaglandins E₁ and E₂⁹, cholera enterotoxin^{10,11} and histamine⁹. PGE₁ and cholera enterotoxin can totally inhibit cytotoxicity, while histamine and the catecholamines are only partially suppressive⁹.

Histamine stimulates adenylate cyclase through a specific receptor, presumably located on the cell membrane, as has been directly demonstrated¹²⁻¹⁴. On pharmacological evidence, Ash and Schild¹⁵ postulated that there are two or more different classes of histamine receptors on cell surfaces, and supporting data have been provided by Black *et al.*¹⁶ using the histamine antagonist burimamide. Antihistamines such as diphenhydra-

mine block what are designated histamine 1 receptor sites, which are responsible for mediating bronchial and ileal smooth muscle contraction, while burimamide blocks the action of histamine in increasing gastric acid secretion and relaxation of the rat uterus, but has little or no effect on histamine 1 activities^{16,17}. Sites antagonized by burimamide have been designated histamine 2 receptor sites.

We have demonstrated that the ability of histamine both to inhibit lymphocyte-mediated cytotoxicity and to augment cyclic AMP levels in murine spleen cell suspensions could be specifically antagonized by burimamide, but not by antagonists of histamine 1 receptors, for example, diphenhydramine¹⁸. This has provided evidence for the concept of histamine receptor units on the membranes of cytolytically active lymphocytes, and has established that these receptors are of the histamine 2 type. As noted, our previous observations have shown only a partial inhibition of cytotoxicity by histamine; moreover, the degree of inhibition noted was more variable from experiment to experiment than that seen with other agents. This suggested to us that there was a relative paucity of histamine receptor sites on cytolytically active cells and that the number of these sites might vary as a function of cellular differentiation. The study reported here was undertaken to follow the appearance of histamine receptor structures on effector T lymphocytes. We found that the susceptibility of cytolytically active cells to suppression by histamine *in vitro* increased markedly with time following primary alloantigenic stimulation. We suggest that this altered susceptibility is due to an increase in the proportion of effector cells bearing histamine receptors.

C57Bl/6 mice were immunized intraperitoneally with 10^7 DBA/2 mastocytoma cells (P815)^{19,20}. At various times following this immunization, groups of immune mice were killed, and single cell preparations made from their spleens²⁰. Mastocytoma cells (carried in ascitic fluid of DBA/2 mice) were collected and labelled with ^{51}Cr -sodium chromate ('Rachromate', Abbott Laboratories, Chicago)¹⁹. Cytolytic activity was assayed by mixing 10^7 splenic lymphocytes with 10^5 ^{51}Cr mastocytoma cells in 1 ml Eagle's minimal essential medium containing 10% inactivated foetal calf serum and 100 IU ml⁻¹ both of streptomycin and penicillin^{19,20}. Incubation was carried out for 4 h at 37° C in an atmosphere of 5% CO₂-95% air. Following incubation, the cells were centrifuged and an aliquot of the supernatant assayed for ^{51}Cr content. The percentage specific lysis was obtained after subtracting the percentage lysis in control tubes containing normal (non-immune) lymphocytes²⁰. The effect of histamine on cytotoxicity was measured by mixing together the lymphocytes, mastocytoma cells, and appropriate concentrations of histamine HCl (Sigma) at the start of incubation. The effect of burimamide (given by Dr J. W. Black, Smith, Kline and French Laboratories, Hertfordshire) on cytotoxicity was studied similarly, by addition of appropriate concentrations of burimamide with or without histamine, at the start of incubation. The percentage inhibition of cytotoxicity in each case was calculated relative to lysis in the absence of drugs⁹.

Cyclic AMP assays were performed by incubating 10^7 splenic lymphocytes, with or without histamine, in 1 ml Eagle's medium containing 10% inactivated foetal calf serum for 10 min at 37° C. The cell suspensions were then centrifuged and the cell pellets resuspended in 0.5 ml 5% trichloroacetic acid. The cyclic AMP content of the resulting suspension was determined by the method of Brown *et al.*²¹.

The inhibition by histamine of the cytolytic activity of lymphocytes obtained from mice immunized 9–17 d previously is shown in Fig. 1. The linear regression line demonstrates that there is a highly significant, positive correlation ($r=0.82$, $P<0.001$) between the time following immunization, and the susceptibility of cytolytically active cells to suppression by histamine. The altered susceptibility to histamine was associated with only a modest change in cytolytic activity. For example, 10 d after antigen administration, the percentage specific cytotoxicity was $37\pm3\%$ (s.e.m.); such cytotoxicity was

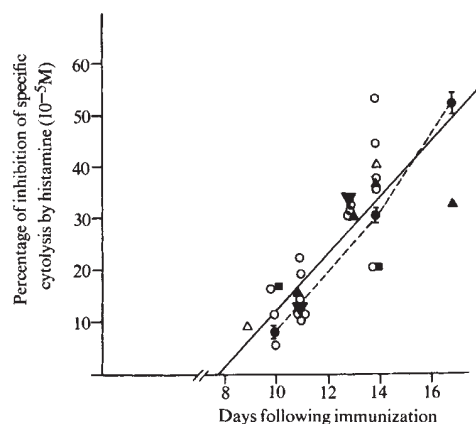


Fig. 1 Adult C57Bl/6 mice were immunized intraperitoneally with 10^7 DBA/2 mastocytoma cells. The cytolytic activity of spleen cell suspensions was assessed at various times thereafter (see text), and the inhibition of cytotoxicity by 10^{-5} M histamine measured on each occasion. Data from thirty experiments are shown together with the linear regression line for all the data. ○, Animals were tested on day one only; in these cases, lymphoid pools from three to ten animals were used. ●, ▲, ▽, Experiments where different animals from simultaneously immunized groups were sequentially followed; in these cases pools of three to five animals were used. In the group of animals represented by (●---●), the mean percentage inhibition of cytotoxicity caused by 10^{-5} M histamine together with the s.e.m. of triplicate assays are shown for 10, 14 and 17 d following immunization. These animals are further described in Table 1.

inhibited only $11\pm2\%$ by 10^{-5} M histamine. Fourteen days after immunization, the percentage specific cytotoxicity was $26\pm3\%$, while inhibition of cytotoxicity by 10^{-5} M histamine had increased to $35\pm4\%$. This phenomenon is further illustrated by the experiments represented as solid circles in Fig. 1. In these studies, animals were immunized simultaneously and then sequentially killed. Inhibition of cytotoxicity by histamine increased throughout the period of study (10–17 d), and was demonstrable with concentrations of histamine ranging from 10^{-7} to 10^{-3} M (see also Table 1).

These experiments were largely performed with pooled cell suspensions obtained from three to ten similarly immunized

Table 1 *In vitro* Effects of Histamine on Cyclic AMP Levels and Cytolytic Activity of Splenic Lymphoid Cells taken at Various Times during the Primary Immune Response to Alloantigen

Days following immunization	% Specific lysis in 4 h	% Inhibition by histamine (molar)					% Increase in cyclic AMP by 10^{-5} M histamine
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
10	29	8	7	8	0	0	$70\pm37^*$
14	32	25	25	30	15	3	52 ± 14
17	24	40	50	52	26	9	48 ± 6
Non-immune	—	—	—	—	—	—	57 ± 12

Thirty C57Bl mice were immunized with DBA/2 cells as described in Fig. 1. Groups of at least six of these mice were killed on each of the days indicated and splenic lymphocytes assayed for cytolytic activity towards DBA/2 target cells and for augmentation of cyclic AMP levels in the presence of histamine. Cytotoxicity and inhibition of cytotoxicity by histamine were calculated as in Fig. 1. For cyclic AMP assays, 10^7 splenic cells were incubated with or without histamine in 1 ml Eagle's medium for 10 min at 37° C. The cells were centrifuged, resuspended in 0.5 ml 5% trichloroacetic acid, and cyclic AMP determined. All assays are based on triplicate or quadruplicate determinations.

* Mean $\pm$ s.e.m. of quadruplicate determinations. These values were computed by randomly pairing values for control and histamine tubes, then taking the mean and s.e.m. of the resulting ratios between these random pairs. This method of calculation yields standard errors considerably larger than those calculated by an alternative approach (that is, computing an average for the control tubes, and calculating the standard error based on variations only in the histamine-containing tubes).

animals. Further studies, in which individual animals were assayed, showed the same pattern and showed very little animal to animal variability. Thus, in one series of experiments (▼, Fig. 1), five animals killed on day 11 and individually assayed showed an inhibition of specific cytotoxicity by 10^{-5} M histamine of $12 \pm 3\%$ (s.e.m.) while five animals from the same group tested on day 13 were inhibited $33 \pm 3\%$ by the same histamine dosage.

The alteration in the susceptibility to histamine was not related to the specific cytolytic activity of the spleen cell populations, for with any given effector cell population the percentage inhibition of specific cytotoxicity by histamine did not change significantly when lymphocyte/target cell ratios were varied from 200 to 1 to 30 to 1, although such alterations changed the total lytic activity about five-fold. Further, when effector cells at the peak of cytolytic activity (day 11) were diluted with normal syngeneic spleen cells to give a lytic activity comparable with later (day 18) cells, the day 11 cells were still only poorly suppressed by histamine (20%), while the day 18 cells with identical cytolytic activity showed marked (43%) suppression.

The variability in histamine inhibition was not due to some unique characteristic of the incubation time employed in the cytolytic assays, for the percentage inhibition of specific cytotoxicity caused by histamine remained constant between 1 h and 4 h after addition of histamine. The increased susceptibility to histamine was likewise not due to an alteration in the threshold histamine concentration which caused inhibition, for maximal inhibition of cytotoxicity was in all cases observed with between 10^{-5} M and 10^{-4} M histamine (Table 1). Failure to inhibit cytotoxicity further with increasing concentrations of histamine suggests that the specific receptor units have been saturated at 10^{-5} M histamine.

It thus seems most likely that the variation in susceptibility to histamine of different cytolytically active lymphoid cell pools relates to the number of lymphocytes bearing specific histamine receptors.

In previous studies we have shown that histamine increases the cyclic AMP levels of spleen cells^{9,18}. If the acquisition of histamine receptors by cytolytically active lymphoid cells reflected a general change in the surface characteristics of splenic lymphocyte populations, one would expect that spleens taken from animals 17 d after immunization would exhibit *in vitro* a greater cyclic AMP response to histamine than would comparable cell populations obtained 10 d after antigen challenge. Consequently, the cyclic AMP levels of spleen cell populations taken from animals at various times following immunization were measured in the presence and absence of histamine (Table 1). The cyclic AMP levels of lymphocytes taken 10, 14 and 17 d after immunization were increased to the same extent by 10^{-5} M histamine in spite of the greatly increased susceptibility to histamine of the cytolytically active cells in the same pool. Similar findings have been obtained with histamine concentrations up to 10^{-3} M. There is, therefore, no evidence of a general change in the proportion of histamine receptor-bearing lymphocytes in the spleen cell pools following immunization. The increase in histamine receptors on effector T cells, which apparently represent only some 2-4% of the total lymphocyte population²⁰, was, as expected, too low to notice by cyclic AMP augmentation criteria.

To ensure that the increase in histamine receptors on cytolytically active cells was a result of acquisition of receptors of the histamine 2 type, the pattern of burimamide antagonism to the histamine suppression was examined. As seen in Table 2, burimamide quantitatively reversed the inhibition of cytotoxicity caused by histamine in a similar manner throughout the period of increasing susceptibility to histamine. This burimamide effect strongly suggests that the histamine receptor which is increasing is of the histamine 2 type. We have observed, however, that burimamide has less effect in reversing the histamine inhibition of cytotoxicity when earlier (10 d) effector cells are used.

Table 2 Histamine Inhibition of Cytotoxicity and its Reversal by Burimamide at Various Stages of the Immune Response

Days following immunization	No. of expts.	% Inhibition of cytotoxicity by 10^{-5} M histamine	% Reversal of histamine inhibition by 10^{-4} M burimamide*
11	8	$16.2 \pm 2.6^\dagger$	44 ± 12
13	3	28.0 ± 3.1	46 ± 1
14	8	32.5 ± 4.1	53 ± 8

* The cytolytic activity of C57Bl/6 animals immunized with DBA/2 cells was assayed, as described in text, at various times following antigenic stimulation. The percentage inhibition in the presence of 10^{-5} M histamine was measured on each occasion. Similarly, the effect of mixtures of 10^{-4} M burimamide and 10^{-5} M histamine on cytotoxicity was also measured. The percentage reversal of histamine inhibition by burimamide was calculated as:

$$100 \times \frac{(\text{percentage inhibition by histamine}) - (\text{percentage inhibition by histamine} + \text{burimamide})}{(\text{percentage inhibition by histamine})}$$

[†] Mean $\pm$ s.e.m.

The immunological significance of lymphocyte sub-populations bearing histamine receptors remains to be established. The recent important observations of Shearer *et al.*¹⁴ would, however, support the concept that such cells may possess an immunoregulatory function. These investigators have shown that when histamine receptor-bearing cells were removed from a normal spleen cell population, the residual cells had an enhanced capacity to produce antibody to sheep erythrocytes. The derivation of the lymphocyte population bearing histamine receptors was not established in Shearer's study. It seems clear, however, that such receptors are not exclusively confined to thymus-derived lymphocytes^{12,13}. Our investigations suggest that the acquisition of histamine receptor units usually follows the differentiation of the antigen recognition unit on the effector T lymphocyte, in that specific cytolytic activity, which is dependent on antigen binding, precedes susceptibility to histamine inhibition. The coincident decline of specific cytolytic activity accompanying the development of histamine receptors makes it tempting to speculate that the histamine receptor unit on effector T cells is related to immunoregulation, perhaps by providing a means of controlling T cell proliferation. We are now investigating this hypothesis.

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¹ Perlman, P., and Holm, G., *Adv. Immunol.*, **11**, 117 (1969).

² Henney, C. S., and Bubbers, J. E., *J. Immunol.*, **110**, 63 (1973).

³ Berke, G., Sullivan, K. A., and Amos, B., *J. Exp. Med.*, **136**, 1594 (1972).

⁴ Henney, C. S., and Bubbers, J. E., *J. Immunol.* (in the press).

⁵ Rosenau, W., in *Cell Bound Antibodies* (edit. by Amos, B., and Koprowski, H.) (Wistar Institute Press, Philadelphia, 1963).

⁶ Bloom, B., *Adv. Immunol.*, **13**, 101 (1971).

⁷ Plaut, M., Lichtenstein, L. M., and Henney, C. S., *J. Immunol.*, **110**, 771 (1973).

⁸ Henney, C. S., and Lichtenstein, L. M., *J. Immunol.*, **107**, 610 (1971).

⁹ Henney, C. S., Bourne, H. R., and Lichtenstein, L. M., *J. Immunol.*, **108**, 1526 (1972).

¹⁰ Strom, T. B., Deisseroth, A., Morganroth, J., Carpenter, C. B., and Merrill, J. P., *Proc. US Nat. Acad. Sci.*, **69**, 2995 (1972).

- ¹¹ Lichtenstein, L. M., Henney, C. S., Bourne, H. R., and Greenough, W. B., *J. Clin. Invest.*, **52**, 691 (1973).
- ¹² Melmon, K. L., Bourne, H. R., Weinstein, Y., and Sela, M., *Science*, **177**, 707 (1972).
- ¹³ Melmon, K. L., Weinstein, Y., Shearer, G. M., Bourne, H. R., and Sela, M., *Israeli J. Med. Sci.*, **8**, 641 (1972).
- ¹⁴ Shearer, G. M., Melmon, K. L., Weinstein, Y., and Sela, M., *J. Exp. Med.*, **136**, 1302 (1972).
- ¹⁵ Ash, A. S. F., and Schild, H. O., *Brit. J. Pharmacol. Chemother.*, **27**, 427 (1966).
- ¹⁶ Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R., and Parsons, E. M., *Nature*, **236**, 385 (1972).
- ¹⁷ Wyllie, J. H., Hesselbo, T., and Black, J. W., *Lancet*, **ii**, 1117 (1972).
- ¹⁸ Plaut, M., Lichtenstein, L. M., Gillespie, E., and Henney, C. S., *J. Immunol.* (in the press).
- ¹⁹ Brunner, K. T., Mauel, J., Cerottini, J. C., and Chapuis, B., *J. Immunol.*, **14**, 181 (1968).
- ²⁰ Henney, C. S., *J. Immunol.*, **107**, 1558 (1971).
- ²¹ Brown, B. L., Albano, J. D. M., Ekins, R. P., Sgherzi, A. M., and Tampion, W., *Biochem. J.*, **121**, 561 (1971).

Inhibition of Histamine Release by Histamine controlled by H2 Receptor

AGENTS which increase the intracellular level of adenosine 3'-5'-monophosphate (cyclic AMP) in human leukocyte preparations such as the catecholamines, methylxanthines and prostaglandins, inhibit the antigen induced, IgE antibody mediated release of histamine from human leukocytes (basophils)¹⁻³. Not only are the dose response relationships for increased cyclic AMP levels and the inhibition of histamine release similar, but the kinetics of the two events are also parallel and it is accepted as a working hypothesis that changes in the cyclic AMP level of human basophils (and mast cells) modulate the *in vitro* allergic response⁴⁻⁶. Recently, we showed that exogenous histamine could increase the cyclic AMP level of human leukocyte preparations and stop the release of endogenous histamine. This occurred at concentrations of histamine (about 10^{-6} M) which are present in the cell suspension under study⁷. For this reason we suggested that histamine could "feedback" to control the spread of an allergic response by decreasing the response of other target cells (tissue mast cells, blood basophils) to an allergenic challenge. No clear effect of antihistamines on the inhibition of histamine release by histamine was observed: concentrations up to 10^{-3} M to 10^{-4} M of several antihistamines had little effect and higher concentrations of these drugs either caused inhibition of histamine release themselves or were cytotoxic^{8,7}.

Ash and Schild have postulated that there are at least two types of histamine receptors. The first, inhibited by standard antihistamines, controlled the increased vaso-

dilatation and smooth muscle contraction caused by histamine. They were designated H1 receptors. Other types of receptors probably mediated the responses not blocked by standard antihistamines, such as gastric acid secretion or the relaxation of the rat uterus⁸. Black *et al.* have validated this supposition by showing that a new drug, burimamide, antagonizes the responses to histamine which are not affected by standard antihistamines (anti-H1) without blocking the H1 receptors⁹. This drug may therefore be considered to combine with and block H2 receptors. We here report that burimamide (anti-H2) blocks the effects of histamine on leukocyte cyclic AMP levels and on the antigen-IgE antibody mediated release of histamine, whereas a standard antihistamine (anti-H1) has no effect.

Human leukocytes from donors allergic to ragweed or grass were separated from the other formed elements of the blood, washed free of serum and suspended in a serum-free Tris buffered medium containing 0.03% HSA, as described previously¹⁰. As histamine, in common with other agents which increase cyclic AMP, has an effect only early in the sequence of reactions leading to histamine release, it was studied in the first stage of the response¹¹. The first stage is generated by exposing the cell preparations to antigen for 2 min at 37° C in the absence of calcium and magnesium and then washing the cells twice in cold buffer. This leads to a cell which is activated and will release histamine (in the second stage) when provided with antigen free buffer containing calcium and magnesium at 37° C. The activated first stage cell is stable for 30 min at 4° C (ref. 11) and the washing and resuspension of the cells take less than 10 min. In these experiments, control cell preparations were exposed to antigen under first stage conditions but the experimental cell preparations were challenged with antigen together with either histamine alone or with the drugs. In other controls, histamine and/or burimamide were studied in the second stage. They had no effect on histamine release. Histamine was measured fluorometrically as previously described^{7,10,11} and cyclic AMP cells were measured by the protein binding method of Brown *et al.*^{12,13} after 10 min incubation with histamine and antihistamines.

Figure 1 shows several experiments comparing the effects of burimamide and diphenylhydramine on the cells of a single donor assayed on successive days. Neither drug had an effect, at these concentrations, on the cells or on the assay of histamine. Burimamide blocked the inhibitory effect of 10^{-6} M histamine about 50% at 10^{-5} M while diphenylhydramine had no effect at this concentration. The relative effects of burimamide and diphenylhydramine were compared in several additional donors with the same result. Four such experiments are shown in Table 1. Several other types of anti-H1 drugs (tripelennamine,

Table 1 Effects of Anti-H1 (Diphenylhydramine) and Anti-H2 (Burimamide) Drugs

A, Blocking of histamine induced inhibition of histamine release

	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	% Inhibition	% Blocking	% Inhibition	% Blocking	% Inhibition	% Blocking	% Inhibition	% Blocking
Histamine, 10^{-6} M	70 ± 2		57 ± 6		42 ± 3		51 ± 3	
Histamine + aH2, 10^{-5} M	33 ± 6	53	14 ± 4	76	14 ± 4	65	12 ± 2	77
Histamine + aH1, 10^{-5} M	75 ± 3	0	52 ± 5	5	46 ± 7	0	51 ± 3	0

B, Blocking of the histamine induced increase in cyclic AMP (pmol cyclic AMP per 10^7 cells)

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
Control	7.8 ± 1.3	1.2 ± 0.7	4.5 ± 0.7	2.8 ± 0.5	3.25 ± 0.3	0.85 ± 0.05
Histamine, 10^{-5} M	14.3 ± 1.7	3.7 ± 0.7	7.8 ± 0.5	4.3 ± 0.9	12.4 ± 2.3	4.2 ± 0.2
aH2, 10^{-4} M	5.5 ± 1.5	2.1 ± 1.1	6.0 ± 0.4	2.5 ± 0.4	—	—
Histamine + aH2	5.4 ± 0.2	1.6 ± 0.5	4.5 ± 0.6	3.0 ± 0.6	—	—
aH1, 10^{-4} M	—	—	—	2.2 ± 0.3	2.0 ± 0.1	0.80 ± 0.6
Histamine + aH1	—	—	—	5.2 ± 0.4	11.8 ± 2.2	4.0 ± 0.6

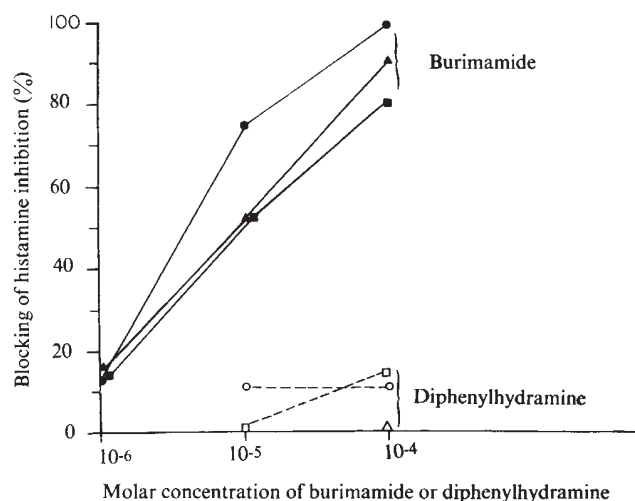


Fig. 1 Blocking of histamine inhibition of IgE mediated histamine release by burimamide and diphenylhydramine.

chlorpheniramine, pyrilamine, cyclizine and promethazine) have also been studied and, at concentrations of up to 10^{-4} M, have no blocking effect on the histamine induced inhibition of histamine release². The effects of burimamide were specific and, in experiments in which it blocked the inhibitory effects of histamine, the inhibition caused by isoproterenol and prostaglandin E₁ was not antagonized.

Burimamide also blocked the increased levels of leukocyte cyclic AMP caused by treatment of the cells with histamine. Six separate experiments are shown in Table 1. In all instances, the effect of 10^{-5} M histamine was blocked by 10^{-4} M burimamide. Diphenylhydramine at this concentration did not block the effect of histamine. As noted, at higher concentrations it has agonist activity and/or is cytotoxic so it could not be used in the present experiments.

These data indicate that the basophils have H₂ receptors for histamine. It would seem that there are at least two receptors which mediate the action of histamine. In the context of allergic or inflammatory responses, the H₁ receptor mediates the vasodilatation and increased vascular permeability which are characteristic of the response to histamine. The H₂ receptor, located on the histamine releasing cells, possibly mediates an inhibitory effect on the inflammatory responses as increased cyclic AMP levels in these cells diminishes histamine release and therefore the intensity of the response. The availability of antagonists for both types of histamine receptors should allow the testing of the relative importance of these two aspects of the inflammatory response in a variety of situations.

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¹ Lichtenstein, L. M., and Margolis, S., *Science*, **161**, 902 (1968).

² Bourne, H. R., Lichtenstein, L. M., and Melmon, K. L., *J. Immun.*, **108**, 695 (1972).

³ Ishizaka, T., DeBernardo, R., Tomioka, H., Lichtenstein, L. M., and Ishizaka, K., *J. Immun.*, **108**, 1000 (1972).

⁴ Lichtenstein, L. M., Henney, C. S., Bourne, H. R., and Greenough, W. B., *J. Clin. Invest.* (in the press).

⁵ Bourne, H. R., Lehrer, R. I., Lichtenstein, L. M., Weissmann, G., and Zurier, R., *J. Clin. Invest.* (in the press).

- ⁶ Orange, R. P., Austen, W. G., and Austen, K. F., *J. Exp. Med.*, **134**, 1365 (1971).
⁷ Bourne, H. R., Melmon, K. L., and Lichtenstein, L. M., *Science*, **173**, 743 (1971).
⁸ Ash, A. S. F., and Schild, H. O., *Brit. J. Pharm.*, **27**, 427 (1966).
⁹ Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R., and Parsons, E. M., *Nature*, **236**, 385 (1972).
¹⁰ Lichtenstein, L. M., and Osler, A. G., *J. Exp. Med.*, **120**, 507 (1964).
¹¹ Lichtenstein, L. M., *J. Immun.*, **107**, 1122 (1971).
¹² Brown, B. L., Albano, J. D. M., Ekins, R. P., and Shgerzi, A. M., *Biochem. J.*, **121**, 561 (1971).
¹³ Gillespie, E., and Lichtenstein, L. M., *J. Clin. Invest.*, **51**, 2941 (1972).

Inhibition and Reversal of Platelet Activation by Cytochalasin B or Colcemid

PLATELETS respond to activation by forming pseudopodia^{1,2} but the relationship between pseudopodia, adhesion, spreading, and aggregation is not known, although it has been suggested that the formation of pseudopodia is an essential condition for platelet aggregation³.

On the basis of our scanning electron microscopy (SEM) and phase contrast microscopy studies of normal platelets which we present here, we postulated that the formation of elongated pseudopodia precedes, and is necessary for, platelet spreading and aggregation. Because platelet pseudopodia contain microfilaments^{4,5}, we suspected that cytochalasin B (CB), a drug which disrupts microfilaments⁶, would inhibit the formation of pseudopodia and would inhibit spreading and aggregation of activated platelets. CB did indeed inhibit the formation of pseudopodia and neither spreading nor aggregation occurred.

In another set of experiments, platelets that already had formed pseudopodia, spread, and aggregated, were treated with CB. After 1 h pseudopodia, spreading, and aggregation were no longer present. We suspected that platelet spreading was dependent on the circumferential band of microtubules that has been observed, just inside the surface membrane, by Hoak⁷. To test this, we used colcemid, a plant alkaloid which binds to microtubules⁸. We found that colcemid blocked membrane spreading but did not inhibit the formation of pseudopodia or platelet aggregation. Further, when platelets that had already spread were treated with colcemid, spreading was no longer present 1 h after treatment.

Plasma rich in platelets was obtained by differential centrifugation of human peripheral blood collected in

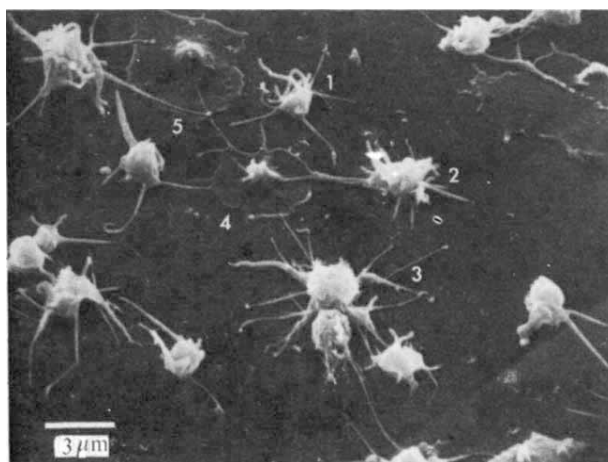


Fig. 1 Activated platelets at different stages after activation.

heparinized syringes. Cells were washed once in Dulbecco's phosphate buffered saline (PBS), resuspended in 25% donor serum and 75% PBS, and 0.1 ml of cell suspension was pipetted onto round glass coverslips. For the cytochalasin B experiments, 0.1 ml $10 \mu\text{g ml}^{-1}$ cytochalasin B (ICL Research Laboratories), dissolved in 0.00001% dimethylsulphoxide (DMSO) in PBS, was added and the cells incubated at 37°C (CO_2 incubator) for 1 h. For the controls, 0.1 ml 0.00001% DMSO without cytochalasin B was added. For the colcemid experiments, 0.1 ml 10^{-6}M colcemid (GIBCO) in PBS was added and the cells incubated for 1 h. The controls had 0.1 ml PBS without colcemid. The platelets were fixed for 30 min in 1% glutaraldehyde followed by 1% osmium tetroxide. The specimens were dried by the critical point method of Cohen⁹ and coated with 50–100 Å of gold in an Edwards Evaporator with a Ladd Rotary Shadower. They were examined in a Kert-Cambridge S4 Scanning Electron Microscope (SEM) operated at 20 kV. Polaroid 55 P/N film was used for the micrographs. A Zeiss phase microscope was used to view cells in both a living and dried state.

Platelets respond to activation (Fig. 1) by forming elongated pseudopodia which adhere to the substrate. After adhesion (1, 2), the pseudopodia thicken as cytoplasm flows into them (3). This continues (4) until spaces between pseudopodia are obliterated and the platelet appears as a central elevation surrounded by a "skirt" of membrane (5). This latter process of membrane flow is termed "spreading".

The aggregation of platelet involves pseudopodia which adhere to other platelets and interlock with pseudopodia of other platelets, thus forming clumps held together by a mesh of pseudopodia (Fig. 2). Usually platelets at the bottom of an aggregate are spread on the glass and the rest of the aggregate is attached to the spread platelets by pseudopodia.

Platelets treated with CB do not form elongated pseudopodia, spread, or aggregate (Fig. 3). DMSO alone does not have this effect. Colcemid treated platelets form pseudopodia and aggregate, but do not spread (Fig. 2).

Platelets that have formed pseudopodia, spread, and aggregated on glass during a 30 min preincubation are treated with CB. After a 1 h incubation, the elongated pseudopodia disappear indicating that formation of pseudopodia is reversible. No spreading or aggregation was observed. Cells preincubated for 30 min on cover slips and then treated with colcemid had pseudopodia and formed aggregates but only 3–5% showed spreading. This suggests that spreading is reversible.

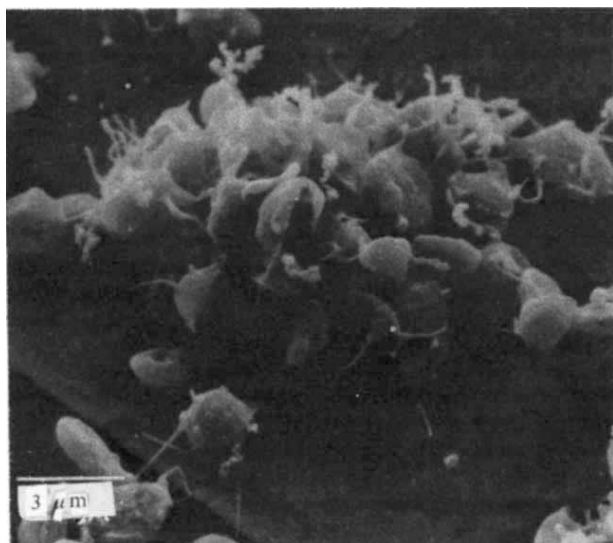


Fig. 2 Aggregate of platelets treated with colcemid.

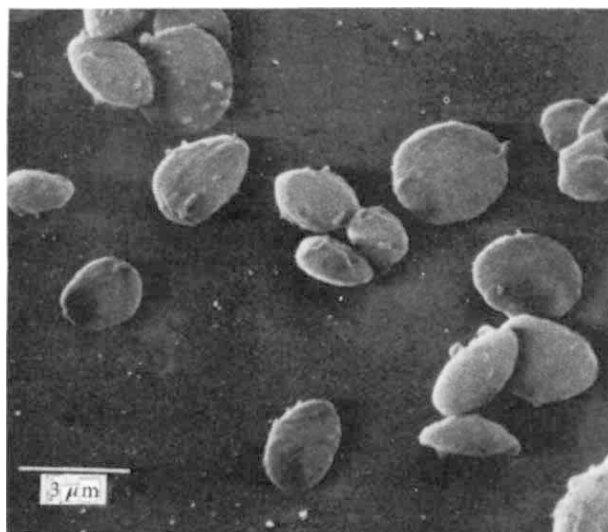


Fig. 3 Platelets treated with cytochalasin B.

These results establish that the formation of pseudopodia precedes and is essential for spreading and aggregation, that spreading is reversible and is not necessary for aggregation, and that adhesion is reversible. Platelet activation may therefore be described as occurring in three sequential stages: pseudopodia formation, adhesion, and spreading and/or aggregation. CB blocks the first step and reverses all the steps if they have occurred before the addition of CB. Colcemid blocks spreading and reverses it. These results indicate that cell to cell adhesion involves microprojections, in this case pseudopodia.

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- ¹ Born, G. V. R., *J. Physiol.*, **209**, 487 (1970).
- ² Born, G. V. R., *Ann. N.Y. Acad. Sci.*, **201**, 4 (1972).
- ³ Barnhardt, M. I., Walsh, R. T., and Robinson, J. A., *Ann. N.Y. Acad. Sci.*, **201**, 360 (1972).
- ⁴ Zucker-Franklin, D., Nachman, R., and Marcus, A. J., *Science*, **157**, 945 (1967).
- ⁵ Zucker-Franklin, D., Nachman, R., and Marcus, A. J., *J. Clin. Invest.*, **48**, 165 (1969).
- ⁶ Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T., and Yamada, K. M., *Science*, **171**, 135 (1971).
- ⁷ Hoak, J. C., *Blood*, **40**, 514 (1972).
- ⁸ Ukena, J. E., and Berlin, R. D., *J. Exp. Med.*, **136**, 1 (1972).
- ⁹ Cohen, A. L., Marlow, D. P., and Garner, G. E., *J. Microscopy*, **7**, 331 (1968).

Properties of Cystinotic Fibroblast-D98 Cell Hybrids studied by Somatic Cell Hybridization

SINCE the discovery of somatic cell hybridization¹ this technique has been used primarily for studies of formal genetics, cell differentiation, and cancer². This study represents an attempt to use this technique to study a metabolic disease in which the primary defect is unknown.

In the nephropathic form of cystinosis, patients appear normal at birth, but by 7–8 months of age they have deficiencies in the renal tubular reabsorption of water, phosphate, sodium, potassium, bicarbonate, glucose, amino acids and other organic

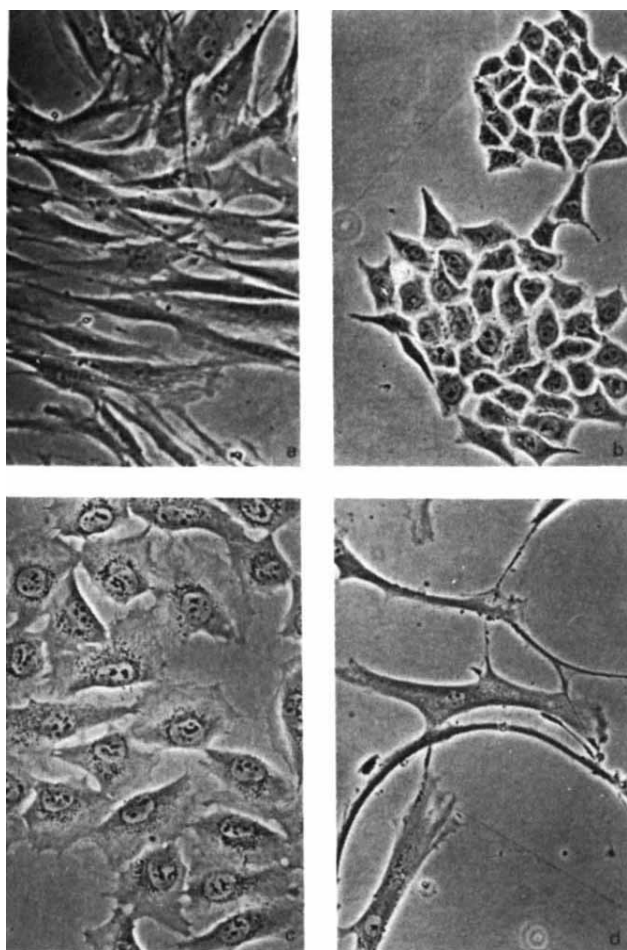


Fig. 1 The cystinotic fibroblasts were obtained from a skin biopsy of a 6-yr-old girl with nephropathic cystinosis. Cultures were maintained in Coon's modification of Ham's F12 medium and hybridization accomplished with β -propiolactone inactivated Sendai virus as previously described¹⁹. All cultures were checked at monthly intervals for evidence of mycoplasma contamination²²⁻²⁴, which was never found. The pictures are phase contrast micrographs ($\times 157$). *a*, Cystinotic fibroblasts; *b*, D98/AH-2; *c*, clone DC 34; *d*, clone DC 26.

acids. This syndrome of tubular dysfunction is called the renal Fanconi syndrome. Although the symptoms caused by these tubular defects are usually easy to manage, children with cystinosis also have progressive glomerular damage, which leads to death from uraemia, generally before 10 yr of age. Most of the body cells in these children contain about 100 times more free (non-protein) cystine than normal. By studying either peripheral leukocytes or fibroblasts grown in culture, it has been possible to identify heterozygotes for this disease, because their cells contain approximately five times more free cystine than normal³⁻⁵.

The discovery that the cystine in cystinotic cells is located in lysosomes^{4,6,7} suggested that the metabolic defect was the absence of a lysosomal enzyme required for cystine metabolism. Attempts at identifying a lysosomal defect, however, have been unsuccessful⁸. The best explanations so far for this disease are either a specific defect of disulphide efflux at the lysosomal membrane or the lack of a reductive mechanism which may or may not be located within lysosomes.

We hoped that hybridizing a fibroblast from a child with cystinosis with a non-cystinotic cell might elucidate the metabolism of cystine in both normal and cystinotic cells⁹. Although it was not possible to predict the free cystine content of such hybrid cells, we hoped segregation would occur and the free cystine content would vary in different hybrid clones. These values might range anywhere from the small amount found in

normal cells to the hundred-fold increase found in cystinotic cells. It might then be possible to correlate these differences with morphological, karyological, or biochemical differences between the hybrid clones.

D98/AH-2 (to be abbreviated D98) is a hypoxanthine-guanine phosphoribosyltransferase negative (HGPRT⁻) derivative of human cell line Detroit 98 (obtained from the American Type Culture Collection, Rockville, Maryland), and was selected as the non-cystinotic cell in this study for six reasons. (1) D98 has a normal cystine content. (2) It is of human origin, and the likelihood of significant chromosome loss is less in intraspecific hybrids². (3) Because it lacks the enzyme HGPRT it is not viable in HAT media (hypoxanthine, aminopterin and thymidine¹⁰), providing an easy selective system against this cell type. (4) This cell, D98, has the A isoenzyme glucose-6-phosphate dehydrogenase¹¹⁻¹³, providing a biochemical marker as the cystinotic cell used would have the B isoenzyme. (5) D98 has a number of marker chromosomes^{14,15}. (6) It is easy to hybridize and has previously been hybridized with cells of murine¹⁶ and human origin^{17,18}. As HeLa cells also have the A isoenzyme glucose-6-phosphate dehydrogenase¹¹ and have marker chromosomes similar to those seen in D98¹⁴, we assume that D98 is a derivative of HeLa cells.

Cystinotic fibroblasts and D98 cells are very different morphologically (Fig. 1*a* and *b*). The cystinotic cells have a typical fibroblastic appearance while the D98 cells are smaller, epithelial in appearance, form more compact colonies, have nuclei which appear larger relative to cell size and contain more nucleoli per nucleus. These two cell types were fused with inactivated Sendai virus by methods previously described¹⁹. Because only a half-selective system was used, the surviving clones included both cystinotic and hybrid clones. Thirteen hybrid clones were selected on the basis of morphology, attempting to obtain as diverse a group of clones as possible. Most clones were epithelial (Fig. 1*c*), but some were fibroblastic (Fig. 1*d*). The clones which were most epithelial also formed the most compact colonies, had the largest nuclei relative to cell size and contained the most nucleoli. Some cystinotic clones were also selected to make certain that treatment with inactivated Sendai virus and exposure to HAT medium had not changed their cystine content.

Table 1 summarizes the chromosome data. D98/AH-2 (D98) is a heteroploid cell line and has a modal chromosome number of 62. If the hybrid cells had no chromosome loss they would have a modal chromosome number of 108 (62+46). It can be estimated from the data in Table 1 that the chromosome loss in these hybrids averaged about 10%. Figure 2 is a karyotype of D98 prepared by Giemsa staining after trypsin treatment.



Fig. 2 Karyotype of D98/AH-2 cell prepared by the trypsin-Giemsa method. The twelve marker chromosomes are labelled m 1 to m 12.

This cell had 63 chromosomes and demonstrated twelve different marker chromosomes. Only one copy of chromosomes 3 and 5 is present. This might suggest that D98 is monosomic for these chromosomes. Closer study of the marker chromosomes showed that this was not the case as portions of chromosome 3 are seen in marker chromosomes 1 and 2, and portions of chromosome 5 are seen in markers 1, 3 and 8¹⁵. Figure 3 is a karyotype of hybrid clone DC 26 (see Fig. 1d) prepared by quinacrine mustard staining. This cell contained 101 chromosomes including one copy each of the twelve D98 marker chromosomes (but four copies of m 8). A more detailed analysis of the chromosomal findings in these hybrids will be reported elsewhere¹⁵.

The cystinotic cell used contained the B isoenzyme G-6-PD. D98 has the faster moving A isoenzyme G-6-PD. All thirteen hybrid clones had both A and B isoenzymes (Fig. 4). When extracts from both parental cells were mixed and electrophoresed together (channel 3, Fig. 4), both the A and B isoenzymes are seen. In the hybrid clones (numbers 2 and 4, Fig. 4) a third band of G-6-PD activity is seen between the A and B isoenzymes. This strongly suggests the presence of



Fig. 3 Fluorescent karyotype of DC 26 cell. It shows 101 chromosomes.

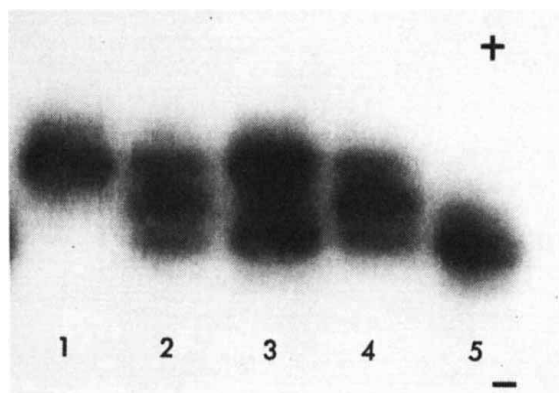


Fig. 4 Electrophoretic pattern of glucose-6-phosphate dehydrogenase (G-6-PD) using vertical starch gel electrophoresis. A Tris-EDTA-borate buffer²⁵ (pH 8.6) was used as outlined by Ruddle and Nichols²⁶. NADP (10 mg) was added to the gel before degassing and to the cathode buffer. Starch gels were run at 0-4° C for 20 h at 7.5 V cm⁻¹ within the gel and approximately 15 mA and were then stained with MTT tetrazolium and phenazine methosulphate. Channel 1 represents parental cell D98; channel 2 represents hybrid clone DC 34; channel 3 represents a mixture of extracts from D98 cells and cystinotic fibroblasts; channel 4 represents hybrid clone DC 26; channel 5 represents cystinotic fibroblasts.

heteropolymeric G-6-PD molecules in the hybrid cells, and provides independent evidence that the cells studied are hybrid cells. The reasoning for this is that the subunits of isoenzymes A and B of G-6-PD are not able to interchange after the complete enzyme molecule is formed, but may do so during their primary aggregation if synthesized in the same cell.

The free (non-protein) cystine content of the hybrid clones is outlined in Table 2. It can be seen that the free cystine content was in the normal range in each hybrid clone studied.

The free cystine content of the hybrid clones is somewhat less than that of the D98 (that is, normal parent) when expressed as nmol half cystine per mg protein. The protein content of a D98 cell is about half that of a hybrid cell, however, and therefore the free cystine content per cell is very similar in the hybrid clones as compared with D98. C-0 is the cystinotic cell before fusion and C-1 is a cystinotic clone which was selected after treatment with inactivated Sendai virus and HAT medium.

Table 1 Karyology of Parent and Hybrid Clones

	No. of metaphases in each range of chromosome counts									Total counted
	<46	46	47-54	55-66	67-80	81-91	92-100	101-112	>112	
"Parent" cells										
D98			1	17		2		2		22
Cystinotic fibroblast	1	9								10
Hybrid cells										
Expected								*		
DC 11					1	3	10			14
DC 14							3	2		5
DC 20						3	5	4		12
DC 22						2	10			12
DC 23					1	5	3	4	2	15
DC 24							12		2	14
DC 26						1	6	8		15
DC 30				1		2	5	4	2	15
DC 31		1				6	8	6	2	22
DC 33							3	4		7
DC 34						1	5	22		28
DC 35A				2		1		6		9
DC 37						1	5	3	1	10

Chromosome preparations and biochemical studies were done within 20-35 generations after the formation of hybrid cells. The cells were treated with colcemid (Grand Island Biological Co., 1×10^{-6} M) for 2-4 h before collection and exposed to 0.075 M KCl for 8 min and fixed in methanol: acetic acid (3:1). The slides were air dried. Quinacrine mustard staining and fluorescent photomicroscopy were performed as described by Caspersson *et al.*²⁰. For demonstration of Giemsa bands, well-dried slides were dipped into 0.2% trypsin solution (Difco Bactotrypsin in isotonic saline) for 15-30 s, rinsed briefly in isotonic saline and stained with Giemsa (Gurr, pH 7.0) for 5-15 min in a modification of the method described by Seabright²¹.

Table 2 Free Cystine Content of Parent and Hybrid Clones

"Parent" cells	(nmol 1/2 cystine mg ⁻¹ protein)
D98	0.11
Cystinotic fibroblasts	
C-0	6.78
C-1	6.05
Hybrid cells	
DC 11	0.02
DC 14	0.04
DC 20	<0.01
DC 22	0.04
DC 23	0.08
DC 24	0.03
DC 26	0.01
DC 30	0.01
DC 31	0.11
DC 33	0.06
DC 34	0.03
DC 35A	0.09
DC 37	<0.01
Mean	0.04

Free cystine was measured as previously described⁶.

The high cystine content of C-1 indicates that the low cystine content in the hybrid clones did not result from these procedures.

Interpretation of these data is difficult. The question to be answered is why none of the hybrid clones was similar to heterozygote cells in its cystine content. The heterozygote cell receives one normal and one abnormal gene and has a cystine content five times greater than normal. The hybrid cell receives two abnormal genes and at least two normal genes and has a normal content of free cystine. Perhaps two normal genes per cell is sufficient even if the cell is very large. Another possibility is that the heteroploid (tumour) cell has taken over "control" of the hybrid cell and that this "control" includes regulation of cystine metabolism.

One further possibility is that the D98 cell makes a substance which can reduce cystine to cysteine in the cystinotic lysosome and thus correct the defect. We are presently pursuing this possibility.

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- ¹ Barski, G., Sorieul, S., and Cornefert, F., *CR Acad. Sci.*, **251**, 1825 (1960).
- ² Ephrussi, B., in *Hybridization of Somatic Cell* (Princeton University Press, Princeton, New Jersey, 1972).
- ³ Schneider, J. A., and Seegmiller, J. E., in *The Metabolic Basis of Inherited Disease* (edit. by Stanbury, J. B.), 3rd ed., 1581 (McGraw-Hill, New York, 1972).
- ⁴ Schneider, J. A., Bradley, K., and Seegmiller, J. E., *Science*, **157**, 1321 (1967).
- ⁵ Schneider, J. A., Rosenbloom, F. M., Bradley, K. H., and Seegmiller, J. E., *Biochem. Biophys. Res. Commun.*, **29**, 527 (1967).
- ⁶ Patrick, A. D., and Lake, B. D., *J. Clin. Pathol.*, **21**, 571 (1968).
- ⁷ Schulman, J. D., Bradley, K. H., and Seegmiller, J. E., *Science*, **166**, 1152 (1969).
- ⁸ Tietze, F., Bradley, K. H., and Schulman, J. D., *Pediat. Res.*, **6**, 649 (1972).
- ⁹ Schneider, J. A., in *Application of Long-Term Lymphocyte Culture to Human Genetics* (edit. by Bergsma, D.), **9**, 160 (National Foundation, White Plains, New York, 1973).

- ¹⁰ Littlefield, J. W., *Science*, **145**, 709 (1964).
- ¹¹ Gartler, S. M., *Nat. Cancer Inst. Monogr.*, **26**, 167 (1967).
- ¹² Steele, M. W., *Biochem. Genet.*, **4**, 25 (1970).
- ¹³ Yoshida, A., Watanabe, S., and Gartler, S. M., *Biochem. Genet.*, **5**, 533 (1971).
- ¹⁴ Miller, O. J., Miller, D. A., Allderice, P. W., Dev, V. G., and Grewal, M. S., *Cytogenetics*, **10**, 338 (1971).
- ¹⁵ Francke, U., Hammond, D. S., and Schneider, J. A., *Chromosoma*, **41**, 111 (1973).
- ¹⁶ Matsuya, Y., and Green, H., *Science*, **163**, 697 (1969).
- ¹⁷ Silagi, S., Darlington, G., and Bruce, S. A., *Proc. US Nat. Acad. Sci.*, **62**, 1085 (1969).
- ¹⁸ Hors-Cayla, M. C., Lyon, G., Heuertz, S., Maroteaux, P., and Frézal, J., *Fourth Intern. Congr. Human Genet.* (abstract, 1971).
- ¹⁹ Schneider, J. A., and Weiss, M. C., *Proc. US Nat. Acad. Sci.*, **68**, 127 (1971).
- ²⁰ Caspersen, T., Zech, L., Johansson, C., and Modest, E. J., *Chromosoma*, **30**, 215 (1970).
- ²¹ Seabright, M., *Lancet*, **ii**, 971 (1971).
- ²² Pollock, M. E., Kenny, G. E., and Syverton, J. T., *Proc. Soc. Exp. Biol. Med.*, **105**, 10 (1960).
- ²³ Hayflick, L., *Texas Rep. Biol. Med.*, **23**, 285 (1965).
- ²⁴ MacPherson, I., *J. Cell Sci.*, **1**, 145 (1966).
- ²⁵ Boyer, S. H., Fainer, D. C., and Naughton, M. A., *Science*, **140**, 1228 (1963).
- ²⁶ Ruddle, F. H., and Nichols, E. A., *In Vitro*, **7**, 120 (1971).

Griseofulvin inhibits Fungal Mitosis

GRISEOFULVIN is an antifungal antibiotic widely used for the treatment of human and animal dermatophytic infections. The *in vivo* antifungal activity of griseofulvin is a reflexion of its peculiar pharmacological properties for, after oral administration, it is specifically localized and concentrated in the keratinized cells of the skin, hair and nails. The invasive growth of dermatophytes parasitizing these tissues is inhibited and the infected tissue is removed by desquamation and the growth of new epidermal layers. Here we report the effects of griseofulvin on fungal mitosis.

Basidiobolus ranarum was used. This has one very large nucleus (25µm long) in each hyphal compartment (Fig. 1). After mitosis, the daughter nuclei move rapidly apart and the compartment is divided by the centripetal growth of a septum which has no central pore. Thus, each hyphal compartment may be regarded as a single uninucleate cell. *B. ranarum* was grown at 30° C in Petri dishes containing 20 ml of a malt extract, yeast extract, glucose, peptone agar medium. The peripheral hyphae



Fig. 1 Phase contrast micrograph of a normal *Basidiobolus* hypha. The large nucleus and nucleolus are easily seen.

of colonies were stained with 0.2% (w/v) aqueous acridine orange for 1 min, washed with water and examined by ultraviolet microscopy. The nucleolus fluoresced red and the condensed mitotic chromosomes fluoresced green. This technique facilitated the recognition of the various stages in mitosis.

A large proportion of hyphae growing on media containing 5 to 10 $\mu\text{g ml}^{-1}$ griseofulvin had compartments which contained more than one nucleus (Figs 2 and 3). Of these multinucleate compartments 88% were binucleate, 9% were trinucleate and 3% were quadrinucleate. The multinucleate condition of many griseofulvin treated hyphae was clearly seen after acridine staining (Fig. 3). In these multinucleate hyphal compartments the nuclear membrane of each nucleus was apparent, showing that each nucleus was physically isolated from the other, even though they were very close together. Control hyphal compartments with two nuclei were seen only very rarely, and if such

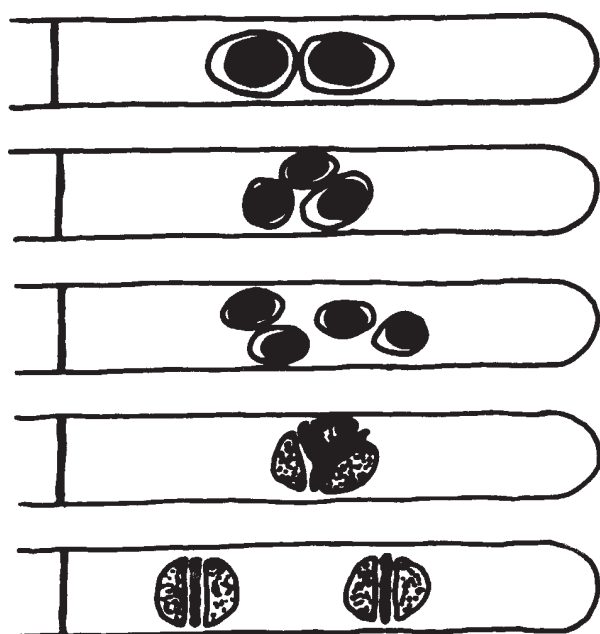
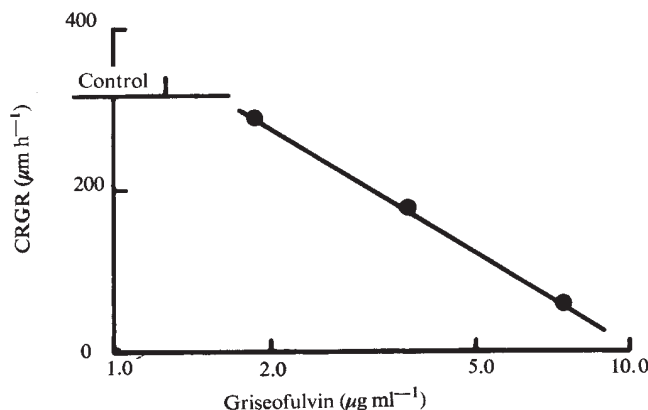


Fig. 2 Illustration of the nuclear abnormalities seen in griseofulvin treated hyphae. Hyphal compartments with two, three or four nuclei were often seen. The Y shaped metaphase often encountered is shown.



Fig. 3 A micrograph of a binucleate hyphal compartment produced by growth of *Basidiobolus* on a medium containing 5 $\mu\text{g ml}^{-1}$ griseofulvin. The micrograph is of material fluorescing after staining with acridine orange. Note the brightly fluorescent nucleoli, the nuclear membranes and the close proximity of the nuclei.



Griseofulvin ($\mu\text{g ml}^{-1}$)	Mitotic index
control	2.4
1.9	4.4
3.75	9.1
7.5	11.3

Fig. 4 The effect of griseofulvin on the colony radial growth rate (CRGR) and mitotic index of *Basidiobolus*. For growth rate experiments increasing concentrations of griseofulvin were incorporated into solid medium and the radial growth rate of colonies measured as previously reported². Mitotic index counts were performed on parallel colonies growing on medium containing griseofulvin. Peripheral mycelium was stained in acridine orange and viewed using ultraviolet illumination on a Zeiss Universal microscope. Mitotic cells were easily seen. 1,000 to 3,000 cells were scanned and those in mitosis were classified into the various mitotic phases.

a cell was seen the two nuclei were always some distance apart. Control hyphal compartments with three or four nuclei were never seen. The presence of multinucleate compartments in griseofulvin treated fungi may explain the previously observed increase in their DNA content relative to controls¹.

Griseofulvin also caused abnormal metaphase configurations, the most usual aberration being a Y shaped metaphase plate (Fig. 2). Occasionally, a griseofulvin treated compartment contained two nuclei which were both in mitosis (Fig. 2). The radial growth rate of *B. ranarum* colonies was inversely related to the logarithm of the griseofulvin concentration in the medium² (Fig. 4). There was, however, a direct relationship between the mitotic index of the mycelium and the griseofulvin concentration (Fig. 4). The known mitotic inhibitors, vinblastine, sodium cacodylate and isopropyl-N-phenyl carbamate (IPC) cause similar effects to griseofulvin on the colony radial growth rate and mitotic index of *B. ranarum*. Neither the colony radial growth rate nor the mitotic index of *B. ranarum* was affected by colchicine or 1,4-dichlorobenzene (two other inhibitors of mitosis). Inhibitors of RNA, DNA and protein synthesis, cell permeability and respiration all caused a reduction in both the colony radial growth rate and the mitotic index of *B. ranarum* (K. G. and A. P. J. T., unpublished). The observed increase in the mitotic index caused by griseofulvin and other mitotic inhibitors was not, therefore, a general response to inhibition of growth.

The number of nuclei in each phase of mitosis was calculated as a percentage of the total number of cells in the population. When these results are plotted as a histogram, a pattern emerges for the treated cells that reflects the point, or points, in the mitotic cycle where the inhibitor has most effect. We have called this histogram a plot of "Phase Accumulation Data". The terms prophase, metaphase, anaphase and telophase are applied to

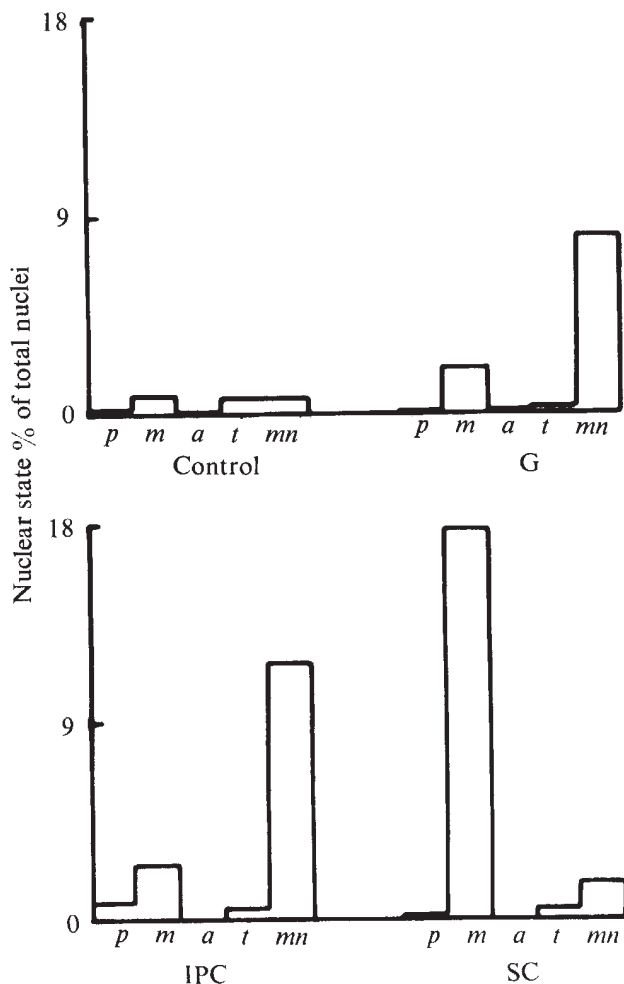


Fig. 5 Phase accumulation data for control colonies and colonies growing on mitotic inhibitors, *p*, prophase, *m*, metaphase, *a*, anaphase, *t*, telophase, and *mn*, multinucleate. The pattern for sodium cacodylate (G) and isopropyl-*n*-phenylcarbamate (IPC) are similar but differ from that of sodium cacodylate (SC). Griseofulvin and IPC increase the number of metaphase nuclei but they also produce large numbers of multinucleate hyphal compartments.

nuclear division in a classical manner. We have used a further term, multinucleate, to indicate a cell containing two or more nuclei each with an interphase appearance. The phase accumulation data, for all the mitosis inhibitors tested, fell into two groups, typified by sodium cacodylate and IPC (Fig. 5). Sodium cacodylate showed a colchicine-like effect in blocking large numbers of cells at metaphase and few multinucleate cells were seen. IPC, however, showed a significant peak at metaphase and very large numbers of multinucleate cells. Figure 5 shows how the phase accumulation data for griseofulvin closely resemble those of IPC but are very different from those of sodium cacodylate.

The nuclei of griseofulvin induced multinucleate compartments were always observed in close proximity (Fig. 3). In such cells the daughter nuclei appeared to move only a very short distance apart after metaphase and then reverted to the early interphase state by re-enclosure in the nuclear envelope. In this sort of situation griseofulvin may act by inhibiting the postulated sliding of microtubules³.

IPC is known to produce disorganization of spindle microtubules in dividing plant cells⁴. Our ultrastructural observations on *Basidiobolus* have shown that griseofulvin produces very similar effects (K. G. and A. P. J. T., unpublished). There is an increasing amount of evidence suggesting that griseofulvin affects microtubules in systems other than fungal hyphae⁵⁻⁸. The ability of griseofulvin to block mitosis, in a manner differing

from that of colchicine, should lead to its use as a valuable research antibiotic.

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¹ Huber, F. M., and Gottlieb, D., *Can. J. Microbiol.*, **14**, 111 (1968).

² Trinci, A. P. J., and Gull, K., *J. gen. Microbiol.*, **60**, 287 (1970).

³ McIntosh, J. R., Hepler, P. K., and Van Wie, D. G., *Nature*, **224**, 659 (1969).

⁴ Hepler, P. K., and Jackson, W. T., *J. Cell. Sci.*, **5**, 727 (1969).

⁵ Malawista, S. E., *J. Cell. Biol.*, **49**, 848 (1971).

⁶ Mauro, F., and Madoc-Jones, H., *Cancer Res.*, **30**, 1397 (1970).

⁷ Nordberg, B., *Scand. J. Haematol.*, **7**, 349 (1970).

⁸ Margulis, L., Neviackas, J. A., and Bannerjee, S., *J. Protozool.*, **16**, 660 (1969).

Antimitotic Action of Griseofulvin does not Involve Disruption of Microtubules

THE antimitotic action of griseofulvin in plant and mammalian cells has been attributed to a colchicine-like disruption of microtubules. Treatment of *Vicia faba*, *Allium* root tips or HeLa cells with griseofulvin, for example, resulted in the accumulation of cells at metaphase¹⁻³. The griseofulvin-inhibited cells contained abnormal arrays of chromosomes, identical to those produced by treatment of dividing cells with colchicine. Using polarization microscopy, Malawista *et al.*⁴ found that the addition of 10^{-5} M griseofulvin to dividing *Pectinaria* oocytes reduced the size of the meiotic spindles, as measured by loss of birefringence. This action was considerably more rapid than with either podophyllotoxin or vinblastine sulphate; moreover, on removal of the griseofulvin, recovery of the spindle also occurred much more rapidly⁴. Recent evidence indicates that several of the drugs which arrest metaphase interfere with microtubule function through different mechanisms. Colchicine and the vinca alkaloids, for example, bind to tubulin (microtubule protein) at different molecular sites^{5,6}. Griseofulvin does not prevent the binding of colchicine to tubulin, nor does it affect the ability of the vinca alkaloids to stabilize the colchicine binding activity of tubulin⁵. This suggests that griseofulvin does not bind at either the colchicine or vinca alkaloid binding sites.

It is now possible to polymerize microtubules *in vitro*, in crude supernatant fractions of brain containing tubulin, by removal of calcium ions by chelation with ethylene bis(oxyethylene-nitrite) tetraacetate (EGTA)^{7,8}. We studied the effect of griseofulvin on this, and found that microtubule polymerization *in vitro* was not prevented. Examination of griseofulvin-inhibited HeLa cells by electron microscopy showed morphologically normal but disoriented microtubules.

Incubation of the crude 30,000g supernatant brain extract containing EGTA at 37° C resulted in the formation of 250 Å diameter microtubules. Addition of 5×10^{-5} M colchicine, 2×10^{-5} M podophyllotoxin, and 2×10^{-5} M vinblastine sulphate or vincristine sulphate immediately before initiation of the polymerization reaction completely prevented microtubule formation. Polymerization *in vitro*, however, was not affected by the presence of griseofulvin (Fig. 1). At the highest griseofulvin concentration tested, 6×10^{-5} M, the microtubules formed were indistinguishable

from those found in untreated samples, and the degree of microtubule formation did not seem to differ significantly.

Inhibition of multiplication of HeLa cells by griseofulvin was concentration-dependent, 50% inhibition being obtained at 4×10^{-5} M griseofulvin, and this concentration producing a mitotic index (c-mitotic cells) of 48% after 30 h of growth (data not shown). Examination of the cells, inhibited in metaphase, showed normal microtubules (Fig. 2). There seemed to be both pole-to-pole and chromosomal microtubules; the latter inserted in the usual manner into the kinetochore, and their numbers were estimated to fall into the normal range. The mitotic centres were of normal appearance, except that there was an occasional increase in the number of small vesicles in the vicinity of the centrioles. The centrioles also showed the usual morphological appearance, and were confined to the mitotic centres. An increase in mitotic centres, however, each containing two centrioles, was observed in many of the drug-treated cells, a finding which may explain the multipolar mitotic patterns observed with the light microscope^{2,3}. The numerous cells found in incomplete mitosis showed mitotic arrest in or after meta-

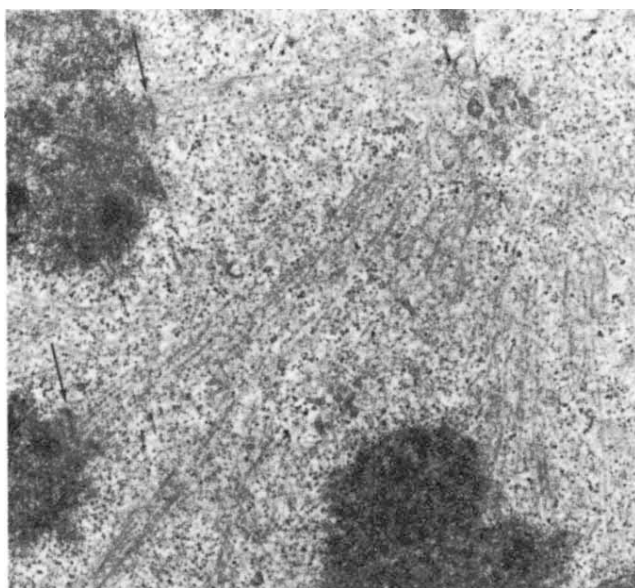


Fig. 2 Section of a HeLa cell blocked in mitosis by exposure to 2×10^{-5} M griseofulvin for 24 h. Normal microtubules radiate from a mitotic centre which occupies the right upper quadrant of the illustration to and past chromosomes. Note the normal attachment of the microtubules to kinetochores (arrows). HeLa monolayer cultures were maintained as described previously^{1,6}. The griseofulvin was dissolved in dimethylsulphoxide (DMSO) so that all cultures (control, griseofulvin, podophyllotoxin, and colchicine-treated) contained final DMSO concentration of 0.1%. Cell growth was not affected by the DMSO. After treatment with the drugs for 6, 12, 24, or 30 h, cells were collected by scraping them off the glass in calcium- and magnesium-free balanced salt solution containing 0.02% EDTA, and mitotic indices and cell numbers were determined. Control and treated cells utilized for electron microscopy were fixed and processed as in Fig. 1. Magnification: $\times 27,500$.

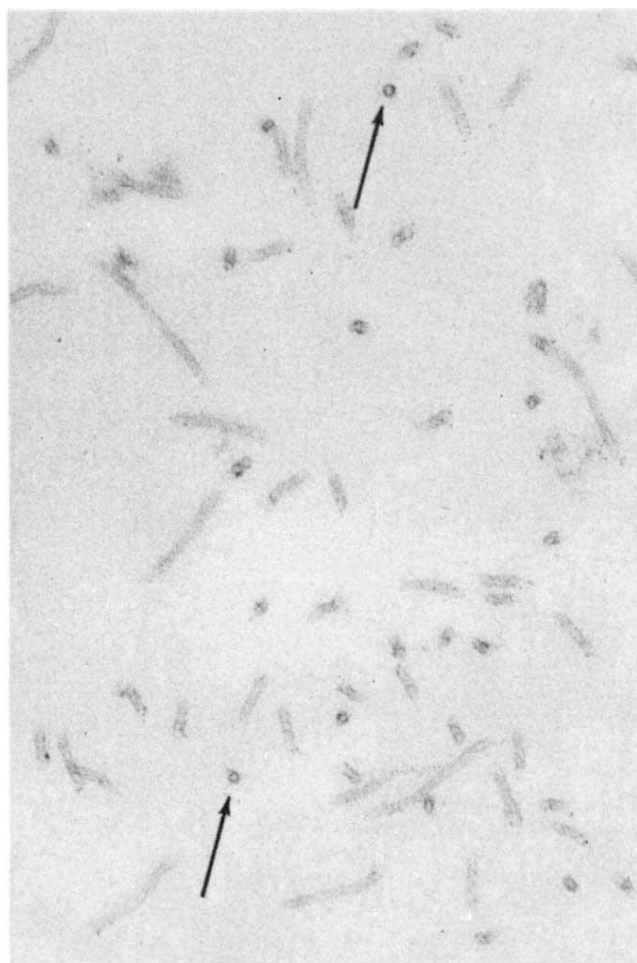


Fig. 1 Microtubules polymerized *in vitro* in the presence of griseofulvin (6×10^{-5} M). The arrows point at cross-sectioned microtubules or 250 Å diameter. *In vitro* polymerization was carried out with 30,000g supernatant extracts of sonicated 13 to 17-d-old chick embryo brains in 20 mM sodium phosphate, 100 mM sodium glutamate, pH 6.75. Extracts contained 0.5 mg ml⁻¹ of tubulin, 1 mM MgCl₂, 1 mM GTP, and 10 mM EGTA. Polymerization was initiated by raising the temperature from 0° to 37° C. The reaction was carried out for 30–60 min. Polymerized microtubules were fixed as a suspension with prewarmed (37° C) buffered 2% glutaraldehyde¹¹. Microtubules were pelleted by centrifugation (10,000g, 30 min), then post-fixed in 1% OsO₄, and embedded in 'Maraglas' by standard procedures^{12,13}. Ultrathin sections were stained with uranyl and lead salts^{14,15}. Magnification: $\times 70,000$.

phase; subhaploid numbers of chromosomes were often found in close apposition to a mitotic centre (Fig. 2). The number of chromosomes associated with any one of the multipolar mitotic centres seemed to be random. HeLa cells, treated with 2×10^{-6} M colchicine or 2×10^{-5} M podophyllotoxin, were arrested at metaphase, and, in contrast to the findings with griseofulvin-inhibited cells, they did not contain microtubules.

Thus, although the action of griseofulvin on mitosis resembles that of other c-mitotic agents such as colchicine, podophyllotoxin, and the vinca alkaloids in a number of ways, it differs in that microtubule assembly is not affected. Griseofulvin shows an effect on mammalian cell mitosis which resembles that of the herbicide isopropyl-*n*-phenylcarbamate (IPC) in plant cells. Treatment of *Hemanthus katherinae* endosperm with IPC resulted in loss of spindle birefringence, and formation of multipolar spindles, and electron microscopy revealed the presence of normal but disoriented microtubules⁹. As with griseofulvin, the microtubules were associated with the kinetochores in a normal fashion (*Hemanthus* does not contain centrioles). Significant differences in the mechanisms of action of these two agents must exist, however, because IPC was found to be totally ineffective in producing mitotic arrest in HeLa cells, even at 10^{-4} M.

How does griseofulvin inhibit mitosis? Although the drug does not affect the assembly of microtubules, it may affect some aspect of microtubule function, possibly a process vital to the sliding of microtubules, which has been proposed to be necessary for separation of chromosomes¹⁰. Alternatively griseofulvin may not affect microtubules directly, but may act on other processes essential for normal completion of cell division. Further understanding of the

mechanism of action of griseofulvin on mitosis may provide new insight into the process of cell division in eukaryotic cells.

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- ¹ Paget, G. E., and Walpole, A. L., *Nature*, **182**, 1320 (1958).
- ² Deysson, G., *Ann. pharm. franc.*, **22**, 17 (1964).
- ³ Deysson, G., *Ann. pharm. franc.*, **22**, 89 (1964).
- ⁴ Malawista, S. E., Sato, H., and Bensch, K. G., *Science*, **160**, 3829 (1968).
- ⁵ Wilson, L., *Biochemistry*, **9**, 4999 (1970).
- ⁶ Bryan, J., *Biochemistry*, **11**, 2611 (1972).
- ⁷ Weisenberg, R. C., *Science*, **177**, 1104 (1972).
- ⁸ Borisy, G. G., and Olmsted, J. B., *Science*, **177**, 1196 (1972).
- ⁹ Hepler, P. K., and Jackson, W. T., *J. Cell Sci.*, **5**, 727 (1969).
- ¹⁰ McIntosh, J. R., Hepler, P. K., and Van Wie, D. G., *Nature*, **244**, 659 (1969).
- ¹¹ Gordon, G. B., Miller, L. R., and Bensch, K. G., *Exp. Cell Res.*, **31**, 440 (1963).
- ¹² Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952).
- ¹³ Freeman, J., and Spurlock, B., *J. Cell Biol.*, **13**, 437 (1962).
- ¹⁴ Sjostrand, F. S., *J. Ultrastruct. Res.*, **9**, 340 (1963).
- ¹⁵ Reynolds, E. S., *J. Cell Biol.*, **17**, 208 (1963).
- ¹⁶ Mizel, S. B., and Wilson, L., *Biochemistry*, **11**, 2573 (1972).

Quantitative Response to Daylength during Diapause in Insects

WITH few exceptions investigations into photoperiodically controlled seasonal activity in insects have used stationary light-dark cycles; no experiments known to us have defined the role of increasing or decreasing daylengths in nature. We therefore investigated the influence of natural daylengths on diapause duration and termination, using the green lacewing, *Chrysopa carnea* Stephens (Neuroptera). This species undergoes a reproductive diapause when subjected to short daylengths below a critical photoperiod¹. A large decrease in stationary daylength above the critical photoperiod can also induce diapause; an increase in stationary daylength below the critical photoperiod can either avert or terminate diapause². It was not clear from these laboratory experiments, however, if *C. carnea* can perceive natural changes in daylength, so we tested (a) whether the naturally decreasing daylengths in autumn have a role in maintaining diapause and (b) whether increasing daylengths after the winter solstice hasten the end of diapause.

To answer the first question we reared *C. carnea* outdoors in Ithaca, NY (42° 27' N). On October 25, approximately three weeks after the animals had reached the full depth of diapause³, we transferred samples from this overwintering population into seven photoperiodic conditions: six with stationary daylengths and one under natural illumination in a heated greenhouse. The animals remained under their respective regimes and were examined daily until diapause ended³ and reproduction began.

The results (curve A, Fig. 1) show (a) that at photoperiods of 13 h or less there is an inverse, linear relationship between diapause duration and photoperiod, and (b) that when the diapause duration of the greenhouse sample (75 d) is plotted against photoperiod (arrow, curve A), a 9.5 h photoperiod is read on the abscissa. As this daylength is equivalent to the

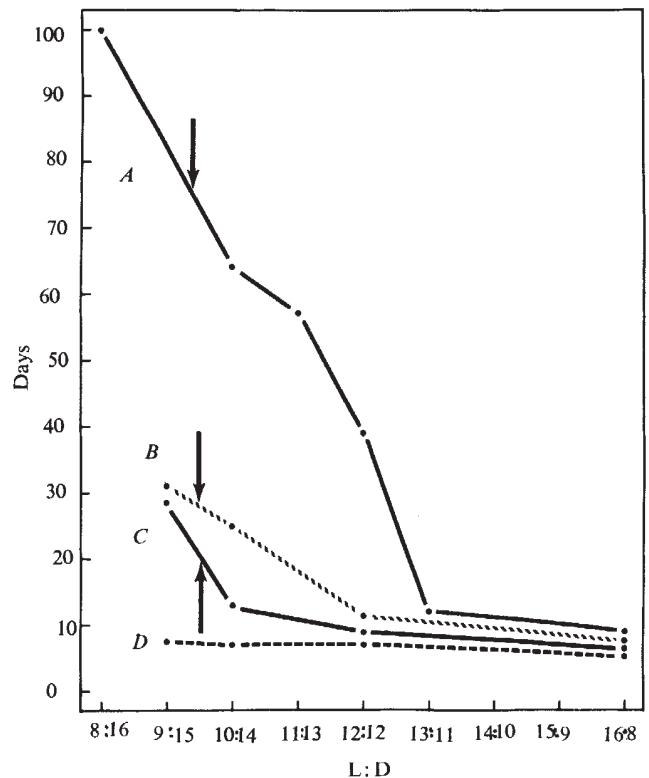


Fig. 1 Response (mean number of days to initiate oviposition) after overwintering *Chrysopa carnea* adults were transferred to various photoperiods. Sample dates: A, October 25, 1971; B, December 22, 1971; C, January 22, 1972; D, February 23, 1972. Arrows, results obtained from groups transferred to the greenhouse on the A, B and C sampling dates. $\bar{x} \pm \text{s.d.}$, sample no. in parentheses: curve A—99.2 $\pm$ 27.8 (8), 64.0 $\pm$ 9.2 (10), 57.0 $\pm$ 10.9 (10), 39.1 $\pm$ 10.1 (12), 11.5 $\pm$ 1.5 (8), 9.0 $\pm$ 0.5 (6), greenhouse (arrow) 75.3 $\pm$ 9.4 (12); curve B—30.7 $\pm$ 6.5 (10), 25.2 $\pm$ 1.5 (8), 12.4 $\pm$ 2.3 (6), 8.3 $\pm$ 0.7 (6), greenhouse (arrow) 27.7 $\pm$ 3.3 (8); curve C—28.6 $\pm$ 10.2 (12), 13.1 $\pm$ 7.3 (12), 8.8 $\pm$ 0.8 (10), 6.6 $\pm$ 0.7 (6), greenhouse (arrow) 20.0 $\pm$ 8.4 (10); curve D—7.6 $\pm$ 0.9 (10), 7.0 $\pm$ 1.5 (10), 6.6 $\pm$ 2.4 (10), 5.8 $\pm$ 0.8 (10). Temperatures: incubators 24° $\pm$ 1° C; greenhouse 23° $\pm$ 4° C.

shortest day of the year at our latitude, it seems that the *C. carnea* perceived and responded to the duration of natural daylengths occurring through autumn and early winter. Thus, as the autumn days became shorter, between the sample date and the winter solstice, the rate of diapause development⁴ slowed. Although, in the laboratory, *C. carnea* is capable of responding to decrease in daylength *per se*, our data here (curve A) indicate that the animals responded quantitatively to the absolute duration of autumn and early winter daylengths. These experiments are the first to demonstrate a quantitative relationship between diapause duration and daylength. In other species it has been shown that the rate of morphogenesis is quantitatively related to photoperiod⁵⁻⁷ and that natural photoperiods may maintain diapause longer than a single stationary short daylength⁸.

Our earlier results have shown that an abrupt increase in daylength, below the critical photoperiod, will break diapause in *C. carnea* in the laboratory² and that chilling does not accelerate diapause development³. To determine whether diapause in this species is terminated by increasing daylengths in nature, we sampled an outdoor diapausing population at the winter solstice and twice afterwards. We transferred samples into various stationary photoperiods and into a heated greenhouse under natural illumination. Our data (Fig. 1) show that within each sample period the various stationary photoperiods produced different responses. Also, the changes in the slopes of curves A, B, C, and D indicate that diapause in *C. carnea* is a dynamic state. Between January 22 (curve C) and February 23

(curve *D*) the response curve flattened out at 6 to 8 d, signifying that diapause had ended in nature during this period.

As diapause in the greenhouse samples (arrows on curves *A*, *B*, and *C*, Fig. 1) persisted as though the animals responded to a stationary photoperiod of approximately 9.5 h of light d^{-1} , we conclude that increasing daylengths after the winter solstice did not accelerate diapause development. Thus, increasing daylengths in nature do not terminate diapause in this species.

Studies attempting to define the role of changing daylengths on diapause fall into three categories: (a) in some species it is not important whether photoperiods increase, decrease, or remain stationary; the only significant factor is whether daylengths are longer or shorter than the critical photoperiod⁹; (b) some species require a change in daylength which crosses a critical photoperiod to evoke diapause, for example, the long-day short-day effect^{10,11}; (c) it seems that some species respond to changes in daylength which do not cross a critical photoperiod. For example, Corbet's experiments¹² suggested that diapause is averted when photoperiods increased by more than a certain increment. Blake^{13,14} infers that decreasing daylengths inhibit pupation, but she did not test the effect of stationary short daylengths. Recently, Vepsäläinen¹⁵ claimed that increasing daylengths avert diapause; his results remain inconclusive because he lacked data from a stationary long day condition for comparison. Our earlier laboratory studies² demonstrated that *C. carnea* can respond to the direction of change in daylengths (both increasing and decreasing) which do not cross the critical photoperiod. Our present results, however, show that in nature it is the absolute duration of the decreasing autumn daylengths which prolongs diapause in this insect. This raises the question whether the insect's photoperiodic clock detects day-to-day differences in absolute daylength or whether it detects larger differences occurring over longer periods.

Our results with *C. carnea* show that natural autumn-winter daylengths may influence diapause induction² and play an important role in diapause maintenance, but that after the winter solstice natural photoperiods do not terminate diapause. In view of these findings (also see Danilevsky *et al.*¹⁶), many laboratory studies of insect dormancy should be re-evaluated, and future studies of diapause induction, maintenance, and termination should take into account the dynamic aspects of dormancy.

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- ¹ Tauber, M. J., and Tauber, C. A., *J. Insect Physiol.*, **18**, 25 (1972).
- ² Tauber, M. J., and Tauber, C. A., *Science*, **167**, 170 (1970).
- ³ Tauber, M. J., and Tauber, C. A., *J. Insect Physiol.*, **19**, 1455 (1973).
- ⁴ Andrewartha, H. G., *Biol. Rev.*, **27**, 50 (1952).
- ⁵ Geyspits, K. F., and Zarakina, A. I., *Ent. Rev.*, **42**, 14 (1963).
- ⁶ Jenner, C. E., unpublished, cited in Corbet, P. S., *A Biology of Dragonflies* (Quadrangle Books, Chicago, 1963).
- ⁷ Kamm, J. A., *Ent. Exp. Appl.*, **14**, 30 (1971).
- ⁸ Lutz, P. E., *A.S.B. Bull.*, **17**, 53 (1970).
- ⁹ Lees, A. D., in *Photophysiology*, 47 (edit. by Giese, A. C.) (Academic Press, New York, 1968).
- ¹⁰ Norris, M. J., *J. Insect Physiol.*, **11**, 1105 (1965).
- ¹¹ Wellso, S. G., and Adkisson, P. L., *J. Insect Physiol.*, **12**, 1455 (1966).
- ¹² Corbet, P. S., *J. Exp. Biol.*, **33**, 1 (1956).
- ¹³ Blake, M., *Nature*, **188**, 168 (1960).
- ¹⁴ Blake, M., *Nature*, **198**, 462 (1963).
- ¹⁵ Vepsäläinen, K., *Ann. Acad. Sci. Fenn. A, IV Biol.*, **183**, 1 (1971).
- ¹⁶ Danilevsky, A. S., Goryshin, N. I., and Tyshchenko, V. P., *Ann. Rev. Ent.*, **15**, 201 (1970).

Changes in Distribution of Electric Charges in the Surface Membrane of Rat Ascites Hepatocarcinoma Cells induced by Con A

SEVERAL kinds of agglutinins of plant origin, such as concanavalin A (con A) or wheat germ agglutinin, agglutinate malignant transformed cells by interacting with specific sites on the surface membrane but only agglutinate normal parent cells after they have been treated with proteolytic enzymes such as trypsin¹⁻³. As malignant transformed cells and normal cells bind almost equal amounts of agglutinins^{4,5}, it has been hypothesized that an increase in susceptibility to the effect of agglutinins may be due to changes in the cell surface such as a redistribution of binding sites⁶ or alterations to the thickness of cell coat material⁷, rather than a simple unmasking of "cryptic" receptor sites^{3,8}. Cell electrophoresis is a suitable method for the detection of minimal changes to the cell surface^{9,10}. Here, we describe the use of cell electrophoresis to determine whether or not any specific changes in the surface of tumour cells associated with agglutination can be induced by the action of con A.

Rat regenerating liver cells and ascites hepatocarcinoma cells (AH 62F and AH 66F)¹¹ were chosen as representative of normal and malignant cells respectively. One millilitre of cell suspension (2×10^6 cells) was mixed with an equal volume of one of various concentrations of con A suspended in physiological saline and was then incubated at 37° C for 10 min (standard condition of contact). The degree of agglutination was determined both macro- and microscopically. After exposure to con A, without further washing, the cells were injected through a polyethylene tube into the visual field of the electrophoretic chamber which had previously been filled with isotonic veronal buffer solution (pH 7.0, ionic strength 0.173). Cell electrophoretic mobilities were determined at $25 \pm 0.2^\circ$ C, using the conventional cylindrical chamber described by Bangham *et al.*¹². Mobilities of twenty or more cells were measured for each sample and a comparison of the mean values between samples was made.

Figure 1 shows that con A induced biphasic changes in the electrophoretic mobility of both of the two strains of rat ascites hepatocarcinoma cells used. Low concentrations of con A ($5\text{--}40 \mu\text{g ml}^{-1}$) increased mobility whereas high concentrations ($100\text{--}300 \mu\text{g ml}^{-1}$) decreased mobility. Both the magnitude of increase in mobility and the concentration of con A needed to cause a decrease in mobility were very different for the two strains of rat ascites hepatocarcinoma studied. Agglutination of cells occurred when concentrations of con A sufficient to facilitate a decrease in mobility were used. These biphasic changes in mobility were scarcely observed when regenerating liver cells were mixed with various concentrations of con A. In this instance a decrease in mobility alone was observed. The biphasic response of mobility took place after tumour cells had been incubated either at 37° C or 4° C for 10 min. Agglutination, however, did not occur at the latter temperature¹³. There was evidence that the biphasic response of the mobility of tumour cells was induced by specific adsorption of con A onto the cell surface. It was completed within several minutes of exposure and remained constant for a further hour. No response was induced by con A which had been previously inactivated by heating at 100° C for 5 min. The response of mobility to the action of con A was reversed by competition with either α -methyl-D-glucopyranoside or D-mannose¹⁴, when added either before or after exposure of tumour cells to con A. For example, the mobility of AH 66F cells had been decreased by $150 \mu\text{g ml}^{-1}$ of con A as much as 11%, whereas the response was completely reversed by further

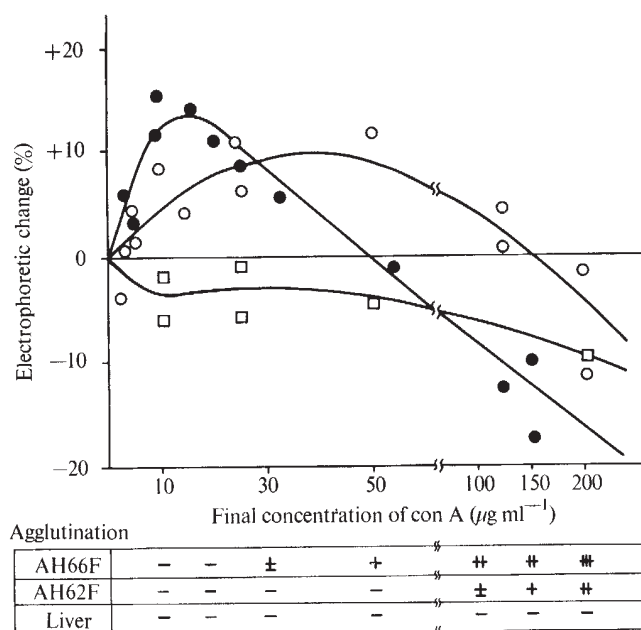


Fig. 1 Changes in cell electrophoretic mobility induced by con A. Two strains of rat ascites hepatocarcinoma cells, AH 66F and AH 62F, were collected from the peritoneal cavities of rats at various intervals during the exponential stage of growth after intraperitoneal inoculation of 10^7 cells. The regenerating liver was collected about 48 h after removing two-thirds of the liver under anaesthesia and individual cells from the liver were obtained by mechanical separation with fine scissors and gentle homogenizing with a loosely fitting 'Teflon' homogenizer. The cells were suspended in physiological saline. Cell electrophoretic mobilities and agglutination were examined after exposure of cells to con A under the 'standard condition of contact'. The degree of agglutination was scored - to + + +. (●), AH 66F; (○), AH 62F; (□), regenerating liver cells.

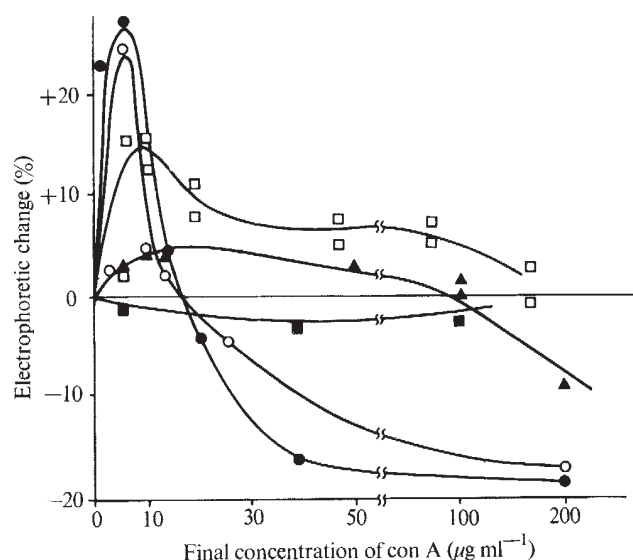


Fig. 2 Changes in cell electrophoretic mobilities induced by con A after treatment with enzymes. Neuraminidase treatment: 10^7 cells in 2 ml of incubation medium containing 0.02 M acetate buffered saline, pH 5.6, and 0.1 mM calcium chloride, were incubated with 10 units of neuraminidase (*Vibrio cholerae*, Calbiochem.) at 37°C for 30 min. Trypsin treatment: 10^7 cells in 2 ml of incubation medium containing 0.1 M phosphate buffered saline, pH 7.0, were incubated with 20 μg of trypsin (Difco Co.) at 37°C for 30 min. Cells treated with enzymes were washed once with saline, and then incubated with con A as standard condition of contact. ●, AH 66F pretreated with neuraminidase; ○, AH 62F pretreated with neuraminidase; ■, regenerating liver cells pretreated with neuraminidase; ▲, AH 66F pretreated with trypsin; □, regenerating liver cells pretreated with trypsin.

washing once with 50 mM α -methyl-D-glucopyranoside or 100 mM D-mannose.

Biphasic changes in the mobility of both strains of ascites hepatocarcinoma cells were amplified by treatment with neuraminidase. When, however, regenerating liver cells were treated with 0.001% trypsin, biphasic changes in mobility similar to those occurring in the ascites hepatocarcinoma cells were induced by con A. The increase in mobility of rat ascites hepatocarcinoma cells induced by con A was slightly inhibited by treatment with trypsin. These results agree with previous reports describing cell agglutination after trypsinization^{3,8} (Fig. 2).

As shown in Fig. 3, ascites hepatocarcinoma cells with markedly increased electrophoretic mobilities at low concentrations of con A ($20\text{--}40\ \mu\text{g ml}^{-1}$) were more susceptible to neuraminidase than were untreated control cells. There was also a noticeable cleavage of sialic acid from cells treated with low concentrations of con A. Heat-inactivated neuraminidase had no effect.

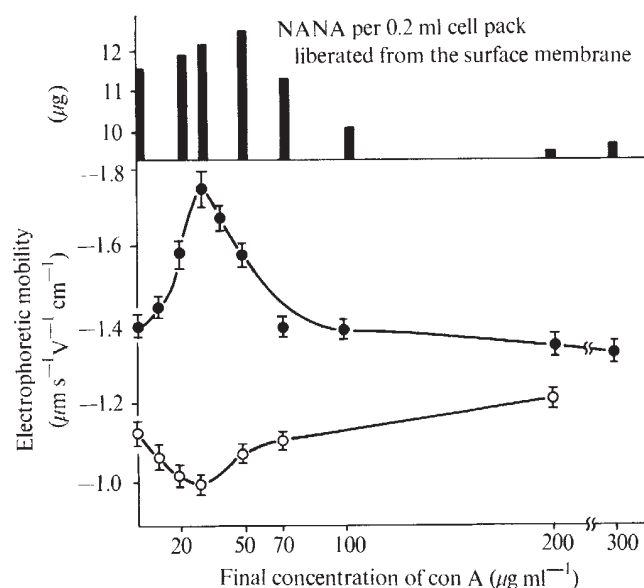


Fig. 3 Effects of neuraminidase on AH 66F cells treated with con A. Packed cells (0.2 ml) suspended in 2 ml of 0.02 M acetate buffered saline (pH 5.6) were preincubated with various concentrations of con A. Ten units of neuraminidase were then added to each cell suspension, which was further incubated at 37°C for 30 min. Just after incubation each sample was centrifuged. The amount of sialic acid in each supernatant obtained was measured by the colorimetric method of Warren¹⁵. A portion of remaining cells, after washing twice by centrifugation with 30 mM α -methyl-D-glucopyranoside and once with physiological saline, was used for the determination of electrophoretic mobility (○). A control experiment was also done with heat-inactivated neuraminidase and without washing treatments (●).

Although Vassar and Culling¹⁶ and Currie¹⁷ independently reported a decrease in the electrophoretic mobility of lymphocytes treated with some phytohaemagglutinins, at rather higher concentrations than those used in the experiment described here, they failed to find concentration-dependent biphasic changes in mobility. We, on the other hand, have found that phytohaemagglutinin (PHA-P; Difco Co.) induces biphasic changes in mobility of AH 66F and Ehrlich ascites carcinoma cells.

Although there may be other explanations, we see the mechanism of the biphasic changes induced by con A as follows. When low concentrations of con A bind to specific sites on the cell surface, the distribution of glycoproteins (and/or glycolipids) is changed. Some of the sialic acid molecules attaching to the terminal position of

saccharide chains and originally situated at deeper sites may shift reactively into the electrokinetic plane of shear of the surface membrane¹⁸, so resulting in an increase in mobility. On the other hand, and in spite of the changes described above, when cells are coated with excess con A, bound con A molecules (Ip 7.1)¹⁹ may cover negative elementary charges of cell surface. They are therefore too far below the new electrokinetic surface, resulting in a decrease in mobility.

We believe the data presented here are concrete evidence of changes in the surface membrane induced by con A, and that increases in mobility which we observed relate to conformational changes in the distribution of electrical charges in the surface membrane. Further investigations are needed to elucidate the correlation between agglutination and changes in the electrophoretic mobility of cells.

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- ¹ Aub, J. C., Tieslan, C., and Lankester, A., *Proc. US Nat. Acad. Sci.*, **50**, 613 (1963).
- ² Burger, M. M., *Proc. US Nat. Acad. Sci.*, **62**, 994 (1969).
- ³ Inbar, M., and Sachs, L., *Proc. US Nat. Acad. Sci.*, **63**, 1418 (1969).
- ⁴ Cline, M. J., and Livingston, D. C., *Nature New Biology*, **232**, 155 (1971).
- ⁵ Ozanne, B., and Sambrook, J., *Nature New Biology*, **232**, 156 (1971).
- ⁶ Nicolson, G. L., *Nature New Biology*, **233**, 244 (1971).
- ⁷ Poste, G., and Reeve, P., *Nature New Biology*, **237**, 113 (1971).
- ⁸ Ben-Bassat, H., Inbar, M., and Sachs, L., *J. Membrane Biol.*, **6**, 183 (1971).
- ⁹ Yamada, T., *Acta Path. Jap.*, **22**, 1 (1971).
- ¹⁰ Yamada, T., Takaoka, T., Katsuta, H., Namba, M., and Sato, J., *Japan J. Exp. Med.*, **42**, 377 (1972).
- ¹¹ Yoshida, T., *Ann NY Acad. Sci.*, **63**, 852 (1956).
- ¹² Bangham, A. D., Flemans, R., Heard, D. H., and Seaman, G. V. F., *Nature*, **182**, 642 (1958).
- ¹³ Inbar, M., Ben-Bassat, H., and Sachs, L., *Proc. US Nat. Acad. Sci.*, **68**, 2748 (1971).
- ¹⁴ Goldstein, I. J., Hollerman, C. F., and Smith, E. E., *Biochemistry*, **4**, 876 (1965).
- ¹⁵ Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).
- ¹⁶ Vassar, P. S., and Culling, C. F. A., *Nature*, **202**, 610 (1964).
- ¹⁷ Currie, G. A., *Nature*, **216**, 694 (1967).
- ¹⁸ Haydon, D. A., *Biochim. Biophys. Acta*, **50**, 450 (1961).
- ¹⁹ Agrawal, B. L., and Goldstein, I. J., *Biochim. Biophys. Acta*, **133**, 376 (1967).

Induction of Mitotic Synchrony by Intermittent Hyperthermia in Ehrlich Carcinoma *in vivo*

THE radiosensitivity of cultured neoplastic cells is generally highest during the mitotic stage of the life cycle^{1,2}; their sensitivity to chemotherapeutic agents is dependent on cell age in the division cycle³⁻⁷. If a method were devised for tumour systems *in vivo* to prolong the time when cells remain in mitosis or to synchronize mitotic activity in a predictable manner, the cell killing efficacy of ionizing radiation might be improved. If the same method could produce increased synchronization in any other phase in which cells are most sensitive to chemotherapeutic agents, an increased efficacy of these drugs might also result². Techniques used *in vitro* to obtain synchronous populations involve selective harvesting⁸, the use of chemicals^{8,9} and the use of temperature¹⁰⁻¹². Most *in vivo* synchronization attempts involve the administration of drugs which disturb cell progression. The pharmacodynamics of such treatments tend to limit severely the degree of synchrony attained. Recently,

33-38% synchrony has been reported for intestinal crypt cells *in vivo*, following mitotic combination drug treatment with arabinosylcytosine and colcemid¹³. Martin and Schloerb¹⁴ increased significantly the mitotic activity of Walker 256 carcinoma cells in rats by intermittently raising the body temperature. If this technique proved to be reliable and the response predictable, the clinical implications are obvious. Radiotherapy during the mitotic interval of a synchronized population could result in increased cell killing with a possible decrease in the total dose, dose per fraction or concomitant number of fractions in a standard radiotherapy regime. These advantages could lead to more effective local tumour control with an increased therapeutic ratio.

Intermittent hyperthermia might also prove useful as a pre-treatment to chemotherapy. Several commonly used anti-metabolites and metabolic inhibitors are phase specific with respect to the cell cycle, with maximum efficacy during the DNA synthesis (S) phase. They include arabinosylcytosine³, hydroxyurea⁴, 2-pyridine carboxaldehyde thiosemicarbazone⁵, methotrexate⁶, 6-mercaptopurine⁶ and 5-fluorouracil⁷. We have evaluated and compared the effects of intermittent hyperthermia on mitotic activity in tumours grown in the solid and ascitic form. Male Balb/c mice 8-10 weeks old (Simonsen Labs, California) were either inoculated intraperitoneally with 0.3 ml ($\sim 2 \times 10^8$ cells) of ascites fluid from a stock mouse carrying a 6-7-d-old Ehrlich ascites tumour or were injected intramuscularly in the left thigh with 0.3 ml of a cell suspension of Ehrlich carcinoma cells.

The heating apparatus consisted of a fully enclosed wire mesh cage with four separate compartments adjustable to variable distances above five 250 W infrared heat lamps. The lamps were connected in parallel to an automatic timer, permitting variability of the length of the heating period and the interval between periods.

To obtain the desired increase in body temperature, we first established the appropriate distance between the lamps and the cage. Mice killed by cervical dislocation immediately after having been heated for varying times and heat intensities had readings taken with a thermister probe inserted through an abdominal puncture. A plateau was reached at 41° C soon after the onset of heating, and this did not change significantly after an hour of heating. Further, the animals tolerated this increased temperature without serious complications. In subsequent experiments thirty-two mice (eight per cage) were intermittently exposed at a chamber height of 15 cm for 96 h. Mice with 2-d-old Ehrlich carcinoma in the ascitic form were subjected to either 14-25 min heating periods at 4 h intervals or 12-30 min heating periods at 8 h intervals. Untreated animals of the same age served as controls. Specimens of the ascites fluid were drawn from the live animals at 1 h intervals immediately following the last heat exposure (96 h after onset). One tumour specimen was obtained per animal. Smears were air dried, fixed in 10% buffered formalin or Carnoy's fixative, hydrated, stained for 11 min in aceto-orcein and counter stained for 30 s with 0.02% fast green¹⁵.

Mice with Ehrlich carcinoma in the thigh with an average tumour diameter of 1.0 to 1.5 cm (approximately 2 weeks after transplantation) were heated for 15 min every 4 h for 96 h (twenty-four treatments in all). Mice were killed by cervical dislocation at 1 h intervals immediately following the last application of heat. The tumours were excised, fixed in Bouin's fixative for one day, dehydrated and embedded in paraffin. Sections, 5 μ m thick, cut tangentially and centrally, were hydrated and stained as previously described with the exception that in the second 95% ethanol rinse, eight drops of concentrated NaH₄OH were used to remove the remainder of the fixative.

In a separate experiment eighteen untreated mice with 6-d-old Ehrlich ascites tumours were aspirated at 4 h intervals beginning at 0900 h to coincide with the time of collection for experimental tumours, accounting for the natural circadian rhythm of tumour cells in untreated animals.

All slides were examined under oil immersion, 1,000 times

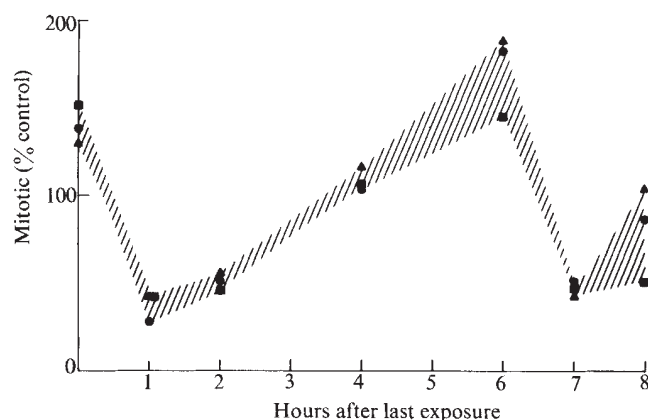


Fig. 1 Relative effect of intermittent hyperthermia on the *in vivo* mitotic index of Ehrlich carcinoma in the solid and ascitic forms. ●, Ehrlich carcinoma heated every 4 h; ▲, Ehrlich ascites heated every 4 h; ■, Ehrlich ascites heated every 8 h.

magnification, with a Zeiss phase contrast microscope. One thousand cells were counted in three separate groups per slide with three experimental slides per time interval after cessation of heat. Cells in late prophase through late anaphase were included for estimating the mitotic index.

Table 1 Mitotic Activity of Ehrlich Carcinoma grown in the Ascitic and Solid Forms in Balb/c Mice following Intermittent Hyperthermia

	Ascites 4 h*	Ascites 8 h†	Solid 4 h*	Controls
0‡	59.0§	68.0¶	62.0	39.7**
1	18.0	18.3	12.7	
2	24.3	20.3	22.7	
4	53.0	47.3	45.3	57.7
6	83.0	65.0	81.3	
7	18.7	20.7	23.3	36.7
8	46.3	23.3	38.7	

* 15 min every 4 h for 96 h.

† 30 min every 8 h for 96 h.

‡ Hours after last heating episode.

§ $\bar{x} \pm 2.6$.

¶ $\bar{x} \pm 2.1$.

|| $\bar{x} \pm 3.2$.

** $\bar{x} \pm 4.7$.

The mitotic indices (mitotic figures per 1,000 cells) found in the three experimental groups and controls are summarized in Table 1, and plotted as a relative function of time after the last exposure to control value (Fig. 1). In the untreated mice mitotic indices of the tumour cells ranged from 39.7 at 0900 h to 57.7 at 1200 h and 37.7 at 1630 h. These values were not significantly different from each other, and the overall average for untreated controls was 44.3 ± 4.7 . In the three experimental groups the mitotic indices were significantly higher than control values at 0 h after the last heat exposure, with values ranging from 59.0 to 68.0.

At 1 h, the mitotic indices in the experimental group varied from 12.7 to 18.3, significantly below the control values. The mitotic indices then increased in all three groups to a maximum at 6 h with values ranging from 65.0 to 83.0. Seven hours after cessation of heat the mitotic indices decreased significantly to values of 18.7 to 23.3 and comparable to the 1 h values. In the present experiments with Ehrlich ascites there was no indication as judged by body weight comparisons of heated and unheated animals that intermittent hyperthermia overtly modified the development of Ehrlich ascites.

The use of high and low temperatures in obtaining mitotic synchronous cell populations has been used for *in vitro* cell systems as well as unicellular organisms such as protozoa¹⁶⁻¹⁹. The present experiment and those by Martin and Schloerb¹⁴ indicate that the maximum synchrony observed occurs 6 h after the last application of whole-body heat. The present study

extends these findings to a different tumour system grown in the ascitic and solid form. The maximum values attained approach more than 180% of control values. This induced synchrony seems to be independent of the heating interval as the results for the 4 and 8 h experiments with Ehrlich ascites cells were essentially the same. It should be noted, however, that in the 8 h group, 6 h after the last heating episode, the increase was 143% that of controls compared with the 4 h group with 187% of controls. Past *in vitro* studies show that hypothermia severely disrupts normal cell cycle progression^{10,17,18}. Similar results are obtained with *in vitro* and *in vivo* systems exposed to prolonged hyperthermia. With chronic hyperthermia there seems to be a block of progression from metaphase to anaphase¹⁷, a change in oxygen uptake¹⁹ and periods of synchrony before reproductive death¹⁹. The actual mechanism(s) involved in inducing partial synchrony with intermittent hyperthermia has not yet been elucidated. The mechanism(s), however, does appear selective for neoplastic tissues^{14,19}.

Studies are in progress using tritiated thymidine pulse-labeling to determine if partial synchrony occurs in the DNA-synthesis phase. Intermittent hyperthermia might shift the tumour cell population into more sensitive phases of the division cycle, enough to increase the anti-neoplastic action of ionizing radiation and chemotherapeutic agents.

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- Elkind, M. M., and Whitmore, G. F., *The Radiobiology of Cultured Mammalian Cells* (Gordon and Breach, New York, 1967).
- Kim, J. H., and Evans, T. C., *Radiat. Res.*, **21**, 129 (1964).
- Karon, M., et al., *Cancer Res.*, **32**, 2612 (1972).
- Krakoff, I. H., Brown, N. C., and Reichard, P., *Cancer Res.*, **28**, 1559 (1968).
- Sartorelli, A. C., Booth, B. A., and Moore, E. C., *Proc. Amer. Assoc. Cancer Res.*, **10**, 76 (1969).
- Philips, F. S., et al., *Cancer Res.*, **33**, 153 (1973).
- Wolberg, W. H., *Cancer Res.*, **32**, 130 (1972).
- Terasima, T., and Tolmach, L. J., *Nature*, **190**, 1210 (1961).
- Hakala, M. T., and Taylor, E., *J. Biol. Chem.*, **234**, 126 (1959).
- Selawry, O. S., *Cancer Res.*, **17**, 785 (1957).
- Kemp, T., and Juul, J., *Arch. Expt. Zellforsch.*, **11**, 602 (1931).
- Wildy, P., and Newton, A. A., *Biochem. J.*, **68**, 14 (1958).
- Verbin, R. S., Diluio, G., Liang, H., and Farber, E., *Cancer Res.*, **32**, 1489 (1972).
- Martin, R. J., and Schloerb, P. R., *Cancer Res.*, **24**, 1997 (1964).
- Conn, H. J., Darrow, M. A., and Emmel, V. M., *Staining Procedures* (Williams and Wilkins, Baltimore, 1960).
- Zeuthen, E., *The Temperature Induced Division Synchrony in Tetrahymena in Synchrony in Cell Division and Growth* (edit. by Zeuthen, E.), 99 (Interscience, New York, 1964).
- Sisken, J. E., and Morasca, L., *Exp. Cell Res.*, **39**, 103 (1965).
- Rao, P. N., and Englebury, J., *Science*, **148**, 1092 (1965).
- Cavaliere, R., et al., *Cancer*, **20**, 1351 (1967).

Differential Effect of Myleran on Two Normal Haemopoietic Progenitor Cell Populations

MANY of the differential effects of cytotoxic agents used in cancer chemotherapy have been attributed to differences between the proliferation rate of normal and tumour cells^{1,2}. When certain closely related tumour cell lines are compared, however, the differences in sensitivity to cytotoxic agents cannot always be accounted for by differences in prolifera-

tion rate and the degree of proliferation dependence of some cytotoxic agents, observed with normal (non-tumour) cells, is frequently insufficient to explain the large difference in sensitivity between normal and tumour cells^{3,4}. It must be presumed, therefore, that there are inherent differences in sensitivity between tumour cell lines and also between tumour cells and normal cells.

The method most widely used for determining the extent to which cytotoxic agents are proliferation dependent *in vivo* is to measure their effect on slowly proliferating stem cells of bone marrow in normal mice and in mice where these cells have been induced to proliferate more rapidly either by prior irradiation⁴, by irradiation with marrow transplantation⁵ or by treatment with endotoxin⁶. The use of a single population of cells to test cytotoxic agents is important because it excludes inherent differences in sensitivity unrelated to cell proliferation rate. We have found that two closely related cell populations of normal haemopoietic tissue have different sensitivities to the di-functional alkylating agent Myleran and that these do not depend on differences in the proliferation rate.

The effect of Myleran (dissolved in dimethylsulphoxide and injected intraperitoneally as an emulsion with arachis oil) on the survival of bone marrow cells, which produce colonies in the spleen following transplantation into heavily irradiated mice⁷, and on the survival of cells which produce colonies when cultured *in vitro* in agar, in the presence of a suitable colony forming stimulating factor⁸, is shown in Fig. 1. The response for each assay was measured 24 h after a single injection.

The survival of agar colony forming cells is considerably greater than the survival of spleen colony forming cells.

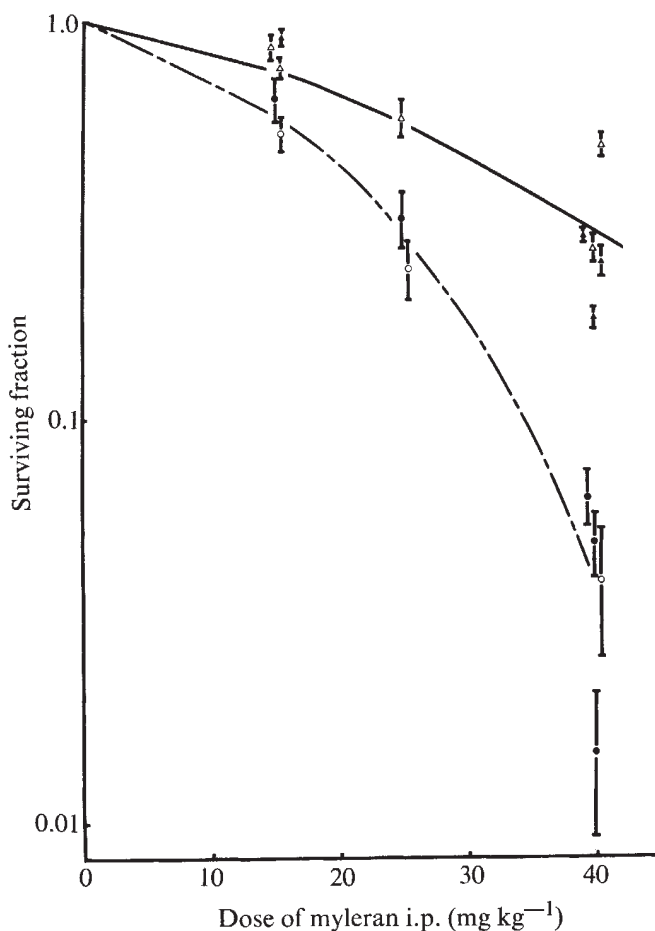


Fig. 1 Survival of spleen colony forming cells from normal (○) or irradiated (●) mice and agar forming colony cells from normal (△) or irradiated (▲) mice after different doses of Myleran.

This is not due to any difference in proliferation rate between the two cell types as the survival curve for each type of cell is unaltered for mice that have received irradiation previously (continuous γ -irradiation at 40 r d⁻¹ for 2 weeks) to increase the rate of proliferation of both cell populations (R. E. M., N. M. B., and S. F. Okell, unpublished).

These results show that, as with tumour cell lines, closely related normal cell populations can also have appreciably different sensitivities to at least one cytotoxic agent and that these are not due to differences in the rate of cell proliferation. This finding may have some relevance to cancer chemotherapy. It is generally considered that the spleen colony forming cells, but not the agar colony forming cells, are the true multipotential stem cells of blood forming tissues. As only the agar colony assay can be employed with human marrow, it is necessary to ascertain in what circumstances the two assays may be expected to be the same or to differ if this assay is to be used as an indicator of the response of the spleen colony forming cells.

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- ¹ Bruce, W. R., Meeker, B. E., and Valeriote, F. A., *J. Nat. Cancer Inst.*, **37**, 233 (1966).
- ² Skipper, H. E., *Cancer*, **21**, 600 (1968).
- ³ Ogawa, M., Bergsagel, D. E., and McCulloch, E. A., *Blood*, **41**, 7 (1973).
- ⁴ Blackett, N. M., and Adams, K., *Brit. J. Haemat.*, **23**, 751 (1972).
- ⁵ Valeriote, F. A., and Bruce, W. R., *J. Nat. Cancer Inst.*, **38**, 393 (1967).
- ⁶ Eaves, A. C., and Bruce, W. R., *Ser. Haematologica*, **2**, 64 (1972).
- ⁷ Till, J. E., and McCulloch, E. A., *Radiat. Res.*, **14**, 213 (1961).
- ⁸ Bradley, T. R., and Metcalf, D., *Austr. J. Exp. Biol. Med. Sci.*, **44**, 287 (1966).

Effect of *Toxoplasma gondii* on the Thymus

It has been shown^{1,2} that rabbits experimentally infected with a virulent strain of *Toxoplasma* develop a severe lymphocytopaenia 3 d after infection and show marked changes in the thymus, chiefly by loss of cortical lymphocytes. In a study of congenital toxoplasmosis in mice, Beverley and Henry (ref. 3 and personal communication) observed depletion of cortical thymus lymphocytes beginning at the age of 5 to 7 weeks and increasing up to 8 to 9 weeks. In mice infected at the age of 8 weeks thymic depletion was pronounced 16 to 30 d after infection but by day 51 the organ was almost completely restored. In the present study we have paid particular attention to the effect of *Toxoplasma* infection in mice during the neonatal period.

CBA and C57Bl mice were infected with *Toxoplasma* of low virulence (strain T626/62) within 24 h of birth. The infectious dose was calculated to contain three to five cysts. During an observation period of 6 weeks, about 25% of the infected animals died, some within the first week but most in the third week, when the infection seems to enter a critical phase.

Groups of five to ten mice were killed at weekly intervals. Total body weight and thymus weight were determined and material collected for histopathology, blood cell counts, serology and isolation experiments.

In comparison with matched controls, growth was significantly retarded in infected animals (Fig. 1). Thymus weights were also markedly reduced in relation to body weights.

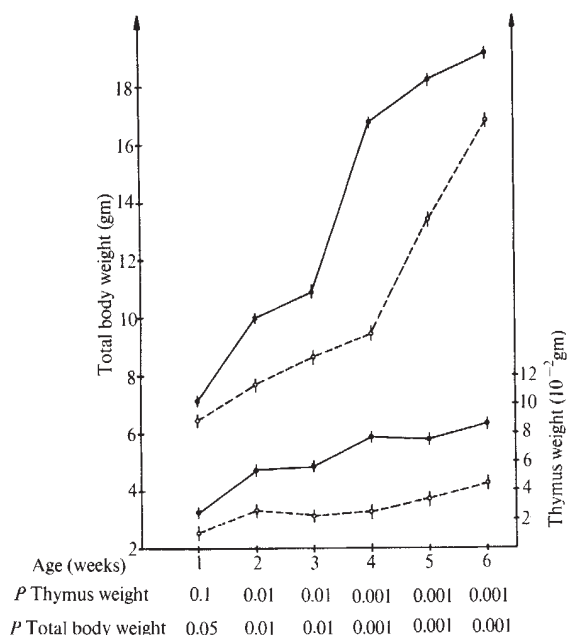


Fig. 1 Total body weight and weight of thymus in mice neonatally infected with low virulence *Toxoplasma* and in normal controls, expressed as means and s.e. for groups of 5–10 mice. —, Normal; ---, *Toxoplasma* infected.

Toxoplasma multiplication was moderate. Only scattered foci, mostly consisting of a single infected cell, were demonstrable by immunofluorescence. Before the age of 3 weeks such foci could be found in at least one of the organs examined (lymphoid organs, liver, lung, brain), but later only sporadically. *Toxoplasmas* were sometimes also found in the medulla or, less often, in the cortex of the thymus. The infected cells were either macrophages or cells of the epithelial reticulum; with the methods used these two types of cell could not be distinguished. Parasites were never found in thymic lymphocytes in sections or imprints. Parasitaemia in low titres was demonstrable in almost all animals during the first 3 weeks and in several mice over the whole observation period.

Depletion of cortical thymocytes was observed as early as the age of 1 week. In pronounced cases, the thymus-dependent zones in lymph nodes and the spleen were also affected. After 4 weeks, foci of large lymphocytes appeared subcapsularly in the thymus and a moderately increased activity of germinal centres in the spleen was observed, possibly indicating regeneration. At no stage was lymphocytopenia recorded. Mice aged 4 and 5 weeks, however, showed increased counts of cells indistinguishable from small lymphocytes. From week 2 a marked monocytosis appeared. The amount of birefringent fat was markedly reduced in the adrenals⁴. Dye test⁵ antibodies were first demonstrable at 3 weeks. In CBA mice high titres were recorded at 6 weeks, whereas in C57Bl the titres remained moderate.

In a second series of experiments immunological reactivity to unrelated antigens was studied. Neonatally infected animals were immunized at 3 or 6 weeks with one dose of either sheep red blood cells (SRBC) or inactivated poliovirus vaccine, and responses measured 4 and 12 d later by spleen cell plaque formation according to Jerne and Nordin⁶ or 21 d later by neutralization tests according to Lagercrantz⁷. Responses to both antigens were significantly reduced, as can be seen from Tables 1 and 2.

In mice infected as adults, a marked effect on thymus weight and immune responses was observed. At 3 weeks after infection a significant reduction in thymus weight was recorded. In a group of animals infected at the age of 8 weeks and examined 3 months later, both size and histology of the thymus

were normal. When the response of these mice to SRBC was tested, however, they were significantly less reactive than age-matched controls (Table 3).

Table 1 Plaque Formation in Normal and Infected Groups of CBA and C57Bl Mice

Experiment No.	Experimental animals	Direct PFC* per 10 ⁵ ± s.e.	t P	Indirect PFC* per 10 ⁵ ± s.e.	t P
1	CBA 3W N†	82 ± 17	3.717	99 ± 13	7.212
	CBA 3W TX‡	16 ± 5	0.001	7 ± 2	0.001
2	CBA 6W N	217 ± 23	5.110	143 ± 21	5.464
	CBA 6W TX	85 ± 11	0.001	16 ± 9	0.001
3	C57Bl 3W N	168 ± 22	7.251	NT	
	C57Bl 3W TX	2 ± 0.7	0.001	NT	
4	C57Bl 6W N	351 ± 76	4.423	NT	
	C57Bl 6W TX	32 ± 12	0.001	NT	

* PFC, plaque-forming spleen cells.

† Normal mice.

‡ *Toxoplasma* infected mice.

Table 2 Neutralizing Antibodies to Killed Poliovaccine in Normal and Neonatally *Toxoplasma* Infected C57Bl Mice. Antibody Level 10 d after Immunization

Experimental animal	Type 1	Type 2	Type 3
Normal	1 100	100	100
	2 400	400	400
	3 400	1,600	400
	4 100	100	100
	5 400	400	400
TX-infected	1 <100	<100	<100
	2 <100	<100	<100
	3 <100	100	<100
	4 <100	<100	<100
	5 ≤100	100	≤100
	6 <100	100	<100

Antibody level 10 d after immunization.

Table 3 Number of Plaque-forming Cells after 5 Months in Normal Mice and Those Infected with Low Virulence *Toxoplasma*

Experimental animals	Direct PFC per 10 ⁶ ± s.e.	t P	Indirect PFC per 10 ⁶ ± s.e.	t P
C57Bl N	204 ± 37	5.032	253 ± 47	5.042
C57Bl TX	15 ± 2	0.001	18 ± 3	0.001

Experiments were also designed to elucidate to what extent stress-mediated corticosteroid activity might be responsible for the thymolytic effect. Mice were adrenalectomized at 5 weeks of age, and 5 d later they were inoculated together with normal controls with either virulent *Toxoplasma* (strain RH) or saline. Animals were killed 24, 48 and 72 h after infection and total body weight, thymus and spleen weights, and blood lymphocyte counts were recorded. The four groups did not show any differences in total body weight.

A progressive loss in thymus weight was observed in infected animals, whether adrenalectomized or not (Fig. 2). A few of the controls, receiving saline only, had slightly smaller thymuses than normal. Spleens were significantly enlarged in all infected animals, equally so in both groups.

Marked peripheral lymphocytopenia was found in all unoperated, infected mice after 48 and 72 h, but in only 3 of 15 adrenalectomized animals.

These experiments show that *Toxoplasma* infection affects both the anatomy and function of the thymus. The mechanism of the effect is obscure, however, although it seems that adrenal activity might be a contributing factor. The observation that adrenalectomy does not eliminate the effect indicates, however,

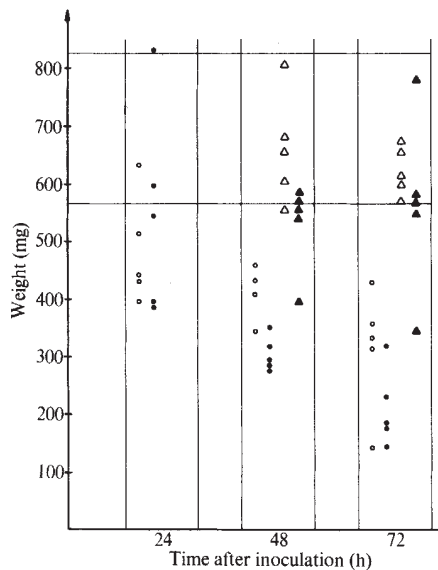


Fig. 2 Weight of thymus in 6-week-old CBA mice, untreated and adrenalectomized, given either 1×10^6 *Toxoplasma* of the virulent RH strain in 0.2 ml saline i.p. or injected with 0.2 ml saline only. Weights at three occasions, 24, 48 and 72 h after infection. Area within horizontal lines represents 95% confidence limits for normal untreated 6-week-old CBA mice. ○, Adrenalectomized, RH infected; ●, normal, RH infected; △, adrenalectomized, saline injected; ▲, normal, saline injected.

that the main cause has to be looked for elsewhere. A direct thymocytolytic effect of the toxoplasms can probably be ruled out, as they were never found in thymic lymphocytes, and experiments on cultured lymphocytes have failed to indicate any direct or indirect lytic effect of the parasite. The possibility of some kind of interference with the differentiation and migration of thymocytes should be considered.

Whatever the mechanisms, the long-lasting depression of immunological reactivity, which follows infection and persists after anatomical restoration of the thymus, might presumably have serious consequences in many situations. Recent publications show that *Toxoplasma* is not unique in this respect. Immunosuppressive effects of malaria⁸, trypanosomes⁹, and *Trichinella*¹⁰ have been reported. It is likely that the widespread parasitic infestation in warm climates might render the population more vulnerable to other infections and account for the relatively poor responses to immunization that have sometimes been reported¹¹.

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- ¹ Hultd, G., *Acta Path. Microbiol. Scand.*, **67**, 401 (1966).
- ² Hultd, G., *Studies on Pathogenesis in Experimental Toxoplasmosis* (Tryckeri AB Balder, 1967).
- ³ Beverley, J. K. A., and Henry, L., *Lyon Med.*, **225**, 883 (1971).
- ⁴ Symington, T., Curris, A. R., Curran, R. C., and Davidson, J. N., *Ciba Found. Colloq. Endocrinol.*, **8**, 70 (1955).
- ⁵ Hultd, G., *Acta Path. Microbiol. Scand.*, **43**, 141 (1958).
- ⁶ Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1965).
- ⁷ Lagercrantz, M., *Acta Path. Microbiol. Scand.*, **79**, 440 (1971).
- ⁸ Greenwood, B. M., Playfair, J. H. L., and Torrigiani, G., *Clin. Exp. Immunol.*, **8**, 467 (1971).
- ⁹ Goodwin, L. G., Green, D. G., Guy, M. W., and Voller, A., *Brit. J. Exp. Pathol.*, **53**, 40 (1971).
- ¹⁰ Faubert, G., and Tanner, C. E., *Exp. Parasitol.*, **30**, 120 (1971).
- ¹¹ McGregor, I. A., and Barr, M., *Roy. Soc. Trop. Med. Hyg. Trans.*, **36**, 364 (1962).

Fatigue of Articular Cartilage

It has been suggested¹ that fibrillation, the earliest change in osteoarthritic cartilage visible to the naked eye, may be the result of fatigue failure. Abnormally high stresses in the superficial layer of cartilage could be produced by unusually high applied loads, incongruity of the joint surface, or softening of the cartilage by mucopolysaccharide loss. Repeated cyclic loading could then lead to fatigue failure in the surface layer. To test this hypothesis post-mortem human articular cartilage was subjected to repetitive compressive loads.

The apparatus was a vertically mounted electromagnetic solenoid, with a hemispherically-ended perspex indenter 3 mm in diameter attached rigidly to its core: weights were attached to the core above the solenoid. Specimens were mounted in a perspex bath containing Ringer's solution plus mycifradin sulphate (0.008 mg l^{-1}) and crystapan benzylpenicillin (0.05 mg l^{-1}). Two electronic timers provided repetitive switching of the supply to the solenoid and the frequency and duration of loading were set to 6 cycles min^{-1} and 1 s respectively. This allowed time for the cartilage to recover after each load application.

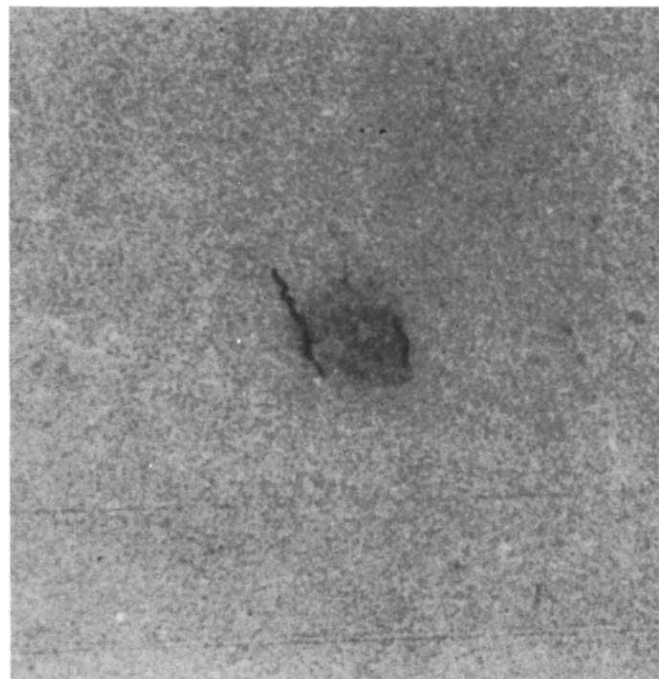


Fig. 1 An area on the superior surface of the femoral head after test, showing surface damage under the indenter.

Figure 1 shows an area of cartilage on the superior surface of the femoral head of a female, aged 47 yr, after 90×10^3 load cycles at a nominal stress of 2 MN m^{-2} . An Indian ink staining technique² was used before and after the test to indicate areas of surface damage. There was no evidence of surface fibrillation before the test. Figure 1 shows the circular area of surface damage under the indenter, as well as two broader markings; Fig. 2 shows that these broader markings have the same orientation as elongated splits produced by pin pricking. The surface damage is identical in overall appearance and orientation to the "minimal fibrillation" observed in osteoarthritic cartilage².

We obtained a measure of the mucopolysaccharide content of the cartilage by the fixed charge density method³. Before fatigue testing, full-depth specimens were removed

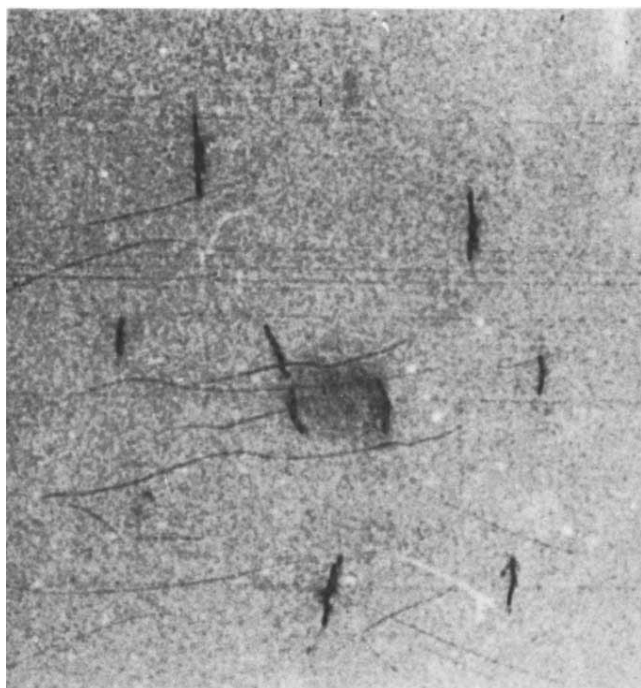


Fig. 2 Same as Fig. 1, but with damaged area surrounded by elongated splits produced by pin pricking.

with a cork borer 3 mm in diameter from two sites remote from the area to be tested and then were stored at -20°C during the test. After testing specimens were similarly removed from the tested area and three other areas. All six specimens were then cut into slices 200 μm thick. Plots of fixed charge density per wet weight of tissue against slice number below the articular surface are shown in

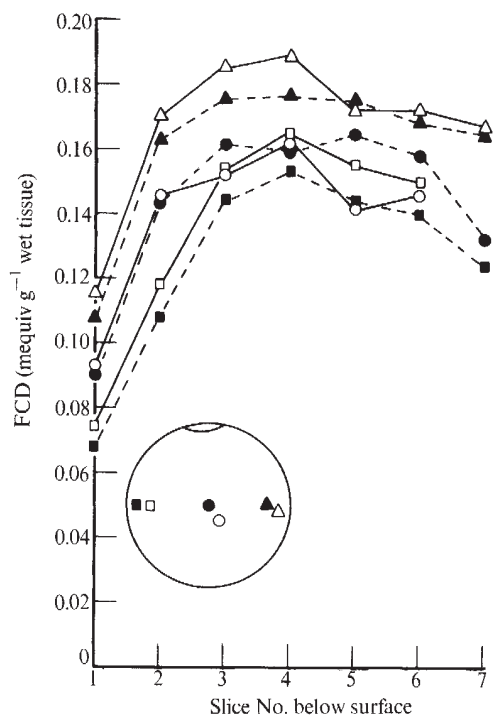


Fig. 3 Fixed charge density (FCD) calculated on a whole tissue basis, against slice number below the articular surface. ■, ▲, Before test controls; □, △, ○, after test controls; ●, fatigue area. Inset represents the sites of FCD specimens on the superior surface of the femoral head.

Fig. 3. The similarity between the profiles of adjacent control areas taken before and after the test shows that there was no general loss of mucopolysaccharides from the femoral head during the test. A comparison of the profiles from the tested area and the adjacent control indicates no loss from the loaded area. All the profiles are typical of completely normal femoral head cartilage⁴. Clearly the surface damage produced during this test was not the result of a loss of mucopolysaccharide from the cartilage, either before the test or as a primary event leading to it.

Although the results of only one test are reported here, other more preliminary experiments (without fixed charge density measurements) have been carried out and in identical condition all the tests produced similar surface damage. We conclude that surface damage of human articular cartilage, similar in nature to early osteoarthritic fibrillation, can be produced by a fatigue mechanism. Further work is in progress to study this phenomenon in greater detail.

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¹ Freeman, M. A. R., *Modern Trends in Orthopaedics* (Butterworths, 1972).

² Meachim, G., *Ann. Rheum. Dis.*, **31**, 457 (1972).

³ Maroudas, A., and Thomas, H., *Biochim. Biophys. Acta*, **215**, 214 (1970).

⁴ Maroudas, A., Evans, H., and Almeida, L., *Ann. Rheum. Dis.*, **32**, 1 (1973).

Monokaryotic Fruiting in the Basidiomycete *Polyporus ciliatus* and its Suppression by Incompatibility Factors

IN most of the higher Basidiomycetes production of fruit bodies is controlled not only by morphogenetic genes but also by incompatibility factors¹⁻³. Generally, the morphogenetic genes can only express their information in matings between monokaryotic strains carrying different incompatibility factors. This leads by nuclear exchange to the establishment of a stable dikaryon and subsequently to the formation of fruiting bodies. In some rare cases, it has been observed that monokaryotic fruiting may be induced either by environmental⁴⁻⁶ or genetic factors, that is, mutation of the incompatibility factors⁷ or of other genes⁸. No definite hypotheses, however, concerning the cooperation of these genetic traits in controlling fruiting body production have been proposed.

We have obtained a stable dikaryotic stock of the wood destroying Basidiomycete, *Polyporus ciliatus* Fr. f. *lepidus* (Fr.) Kreisel, which forms fruiting bodies containing numerous four-spored basidia, when grown on corn meal agar, containing 3% malt extract, after 20 d at 25°C (Fig. 1). Oidia or other cells of vegetative propagation are not produced. Using the method described for Ascomycetes⁹ we have isolated 100 basidiospores; from these, 85 spores germinated. Surprisingly, all of the resulting mycelia developed various kinds of stomatic structures which were more or less similar to normal fruiting bodies (Fig. 1). Approximately 70% of these abnormal fruiting bodies had hymenia. The basidia carried predominantly only two spores.

Cytological observations have revealed that these spores contained only one nucleus and the mycelia do not form

clamp connexions. An inoculation of basidiospores from all monokaryotic fruitings unequivocally gave rise to the same phenotype. We conclude that these abnormal fruiters are true monokaryotic mycelia.

Testing these monokaryons in all possible combinations, we found, as expected³, four mating types (A_1B_1 , A_2B_2 , A_1B_2 , A_2B_1) which reacted according to the tetrapolar mechanism of the homogenic incompatibility system in the same way as known in other Basidiomycetes. This means that clamp connexions and normal fruiting bodies occurred only when A and B factors of both partners were non-identical. Common A or common B factors led to the typical "flat" or "barrage" reaction.

In the offspring of the compatible crosses we found the parental phenotypes and also new phenotypes which apparently arose by recombination. Complete genetic analysis of the monokaryotic fruiters, now in progress, should establish a variety of different genes, each of which are part of a sequence controlling the morphogenetic procedures leading to the differentiation of fruiting bodies. A similar sequence is already known for the Ascomycete *Sordaria*¹⁰.

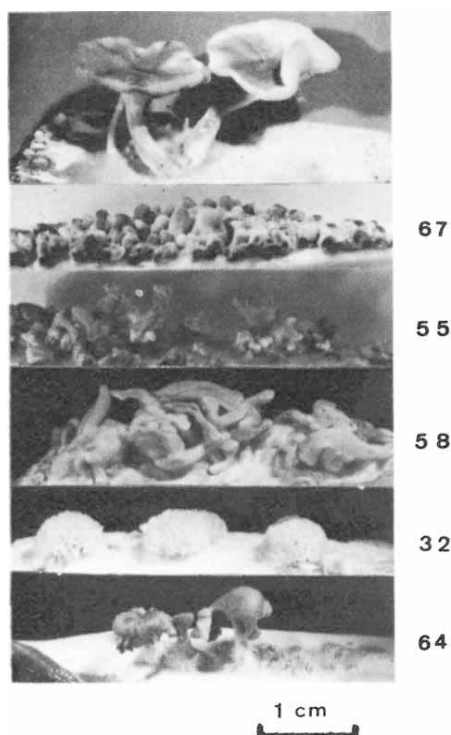


Fig. 1 Various types of fruiting bodies of *Polyporus ciliatus*. Normal fruiting bodies from the dikaryotic stock (top); abnormal fruiting bodies from monokaryotic strains, marked by their isolation numbers (below).

During the crossing studies between the monokaryotic fruiters a new phenomenon was observed. In all crosses in which nuclear migration occurred (that is, unlike A and B, common A unlike B) the monokaryotic fruiting was inhibited as soon as the dikaryon or heterokaryon was established. Neither of the mating partners forms any kind of abnormal fruiting bodies in these regions which have become di or heterokaryotic (Fig. 2). As nuclear migration does not occur in common B combinations, no inhibition of monokaryotic fruiting was observed. The same holds true for combinations between like mating types which in spite of common A and B factors have become heterokaryotic. We conclude that the action of the genes responsible for the monokaryotic fruiting is suppressed as soon as nuclei with

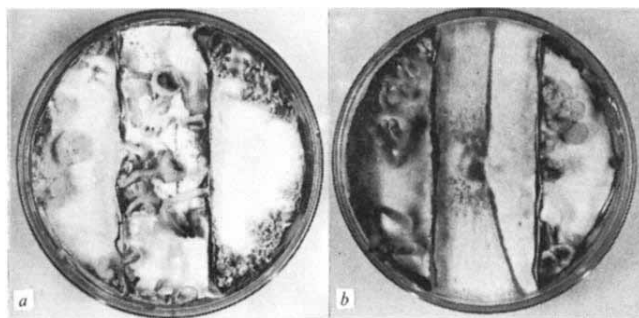


Fig. 2 Crosses of monokaryotic strains. *a*, Cross between strain 70 and 72 being compatible for both mating type factors. After inoculation of the dish the lateral parts of both partners have been dissected with sterile aluminium foil in order to avoid nuclear migration. Whereas the middle section has become dikaryotic and forms unequivocally normal fruiting bodies, both of the lateral sections show monokaryotic fruiting. *b*, Cross between strain 63 and 64 having common A and different B factors. Arrangement as in (*a*). As nuclear migration has taken place in the middle section, there is no fruiting body production, whereas in the lateral sections monokaryotic fruiting occurs.

different incompatibility factors are in a common cytoplasm, regardless of whether normal fruiting bodies are formed (for example, $A_1B_1 \times A_2B_2$) or only sterile heterokaryons occur (for example, $A_1B_1 \times A_1B_2$). The original laboratory stock can therefore be considered to consist of a population of genetically different nuclei, each of which is unable to express its genotype unless the incompatibility factors present in the same cytoplasmic environment are identical and thus do not exhibit any suppressing activity. The regulating effect of the incompatibility factors is not only restricted to the first morphogenetic steps leading to the formation of a stable dikaryon with clamp connexions. The subsequent morphogenetic procedures which end with the formation of fruiting bodies are also controlled by these factors.

Our data, the details of which will be published elsewhere, are not only an example of cooperation between incompatibility factors and morphological genes, but will help to explain the phenomenon of morphogenetic differentiation. In addition, the suppression phenomenon may be used as a model to explain by a genetic mechanism the phenotypic balance of a population consisting of numerous genetically different nuclei.

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Received March 12; revised April 10, 1973.

- ¹ Raper, J. R., *Genetics of Sexuality in Higher Fungi* (Ronald Press, New York, 1966).
- ² Esser, K., and Kuenen, R., *Genetics of Fungi* (Springer-Verlag, Berlin, Heidelberg, New York, 1967).
- ³ Esser, K., *Handbuch der Pflanzenphysiologie*, **18**, 321 (Springer-Verlag, Berlin, Heidelberg, New York, 1967).
- ⁴ Niederpruem, D. J., Hobbs, H., and Henry, L., *J. Bact.*, **88**, 1721 (1964).
- ⁵ Leonard, T. J., and Dick, S., *Proc. US Nat. Acad. Sci.*, **59**, 745 (1968).
- ⁶ Uno, I., and Ishikawa, T., *Molec. Gen. Genet.*, **113** (1971).
- ⁷ Raper, J. R., and Krangel, G. S., *Mycologia*, **50**, 707 (1958).
- ⁸ Leonard, T. J., and Raper, J. R., *Science*, **165**, 190 (1969).
- ⁹ Esser, K., *Neurospora Newsletter*, **15**, 27 (1969).
- ¹⁰ Esser, K., and Straub, J., *Z. Verebungsl.*, **89**, 729 (1958).

Shared Antigens of Parasitic Nematodes and Host Plants

ANTIGENS which metazoan parasites share with their warm blooded hosts have been known for many years. Examples of other "common antigens" can also be found among the viruses, protozoa, bacteria and their respective hosts. The occurrence and significance of this phenomenon has been the subject of a comprehensive review¹.

It has been demonstrated that immunological cross reactions occur between pathogens of plants and their hosts. Thus *Verticillium albo-atrum* possesses antigenic components found in cotton plants and antisera to *Ustilago maydis* cross react with antigens prepared from corn². Some phyto-bacteria and their hosts also have antigens in common³.

Wimalajeewa and DeVay⁴ have shown that a common antigen relationship is a feature of compatible host-pathogen interactions. Host susceptibility may be dependent, therefore, upon successful molecular mimicry by the parasite. Other phenomena which may involve immunological cross reactions include species competition whereby the presence of one species is inimical to a second species⁵ and the evolution of host specific strains in which morphologically identical organisms differ in host preference.

Plant parasitic nematodes and their hosts offer a unique model for the study of immunological cross reactions between host plants and metazoan pathogens. Nematodes such as the root-knot nematode, *Meloidogyne incognita*, live most of their lives in intimate association with host tissues, stimulating neoplastic growth of the host and developing a nutritive relationship essential for the survival of the parasite. Successful establishment of a parasitic mode should therefore indicate convergent evolution of antigenic components. Molecular mimicry in plant parasitic nematodes would be remarkable, however, in that serologically similar proteins and other antigens are not known to occur in plant-animal relationships. To our knowledge, this communication is the first confirming their existence.

Eggs and larvae of *M. incognita* were collected from infected chili pepper, *Capsicum frutescens*, and pathogenicity of the larvae was tested on cotton, *Gossypium hirsutum*. For immunization of rabbits, surface sterilized eggs and freshly hatched, surface sterilized larvae were homogenized in a French pressure cell with 0.005 M phosphate buffer containing 0.001 M MgCl₂. Whole homogenates were used for immunization of rabbits and supernatants, following centrifugation (10,000g for 20 min) for immunodiffusion tests. Seeds of cotton and soybean, *Glycine max*, were germinated aseptically and, after 3 d of growth, the terminal 7–8 cm of root tip was excised and prepared⁴ for immunization or use in immunodiffusion tests.

Antisera for plants and nematodes were produced in New Zealand white doe rabbits with a schedule consisting of intravenous, intraperitoneal and intramuscular injections over a 14 d period⁴. Antisera also were produced by three foot-pad injections given at 2 week intervals⁶. In homologous reactions, the agglutinin titres of antisera for eggs and larvae of the nematode were between 6,400 and 25,600 and for the host plants between 800 and 12,800.

Serological reactions were conducted in Ouchterlony⁷ agar-gel diffusion plates. Antisera for eggs of *M. incognita* cross reacted with antigens from both hosts, cotton and soybean (Fig. 1) as did larval antisera. Antisera for cotton and soybean roots also cross reacted with antigens from larvae and eggs. In all cross reactions, at least one precipitin band was formed. In the case of cross reactions involving egg antisera and antigens from cotton, two precipitin bands were formed, only one of which was shared with soybean. These common antigens seemed

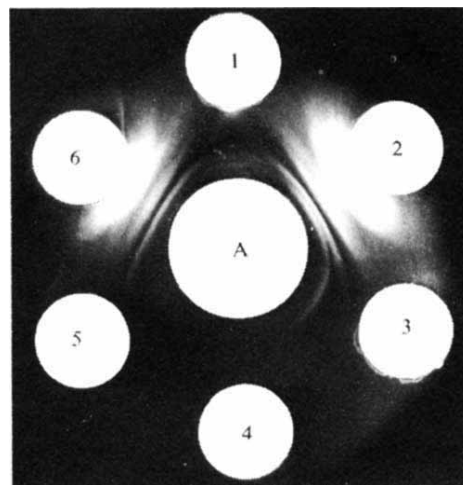


Fig. 1 Precipitin patterns in agar double-diffusion plates. The centre well (A) contained antiserum for eggs of *Meloidogyne incognita*. Wells 2 and 6 (1/2 dilution) contained homologous antigen and wells 1 and 3 antigens from cotton and soybean respectively. Well 4 contained buffer and well 5 1/4 dilution of cotton antigen.

to be serologically related to each other as the precipitin bands joined completely in agar gel diffusion tests. None of the antigenic preparations reacted with normal sera.

Reports on the presence of an immune response in plants are not convincing^{8–11}. While the presence of phyto-precipitin has been shown¹² the chemical composition and antigenic specificity of these substances have not been studied. The susceptibility of plants to infections cannot yet be explained in terms of immunological tolerance such as those suggested for animal-host systems. The occurrence of common antigens between plant parasitic nematodes and their hosts not only raises many interesting questions regarding the specificity of the host-parasite relationship, host resistance, nematode pathotypes, and so on, but reflects upon the origins of phyto-parasitism itself. Should common antigens represent structural or functional components, it would not seem surprising that parasitism in the higher nematodes (Tylenchida) seems to be almost as old as the origin of higher terrestrial plants¹³. If plant parasitic nematodes evolved from fungal feeding ones, further models for phylogenetic investigations using the common antigen approach are possible, because the latter are among the most abundant of the soil-dwelling Nematoda.

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- ¹ Damian, R. T., *Amer. Natur.*, **98**, 124 (1964).
- ² DeVay, J. E., Charaduttan, R., and Wimalajeewa, D. L. S., *Amer. Natur.*, **106**, 185 (1972).
- ³ Schnatehorst, W. C., and DeVay, J. E., *Phytopathology*, **53**, 1142 (1963).
- ⁴ Wimalajeewa, D. L. S., and DeVay, J. E., *Physiol. Plant Pathol.*, **1**, 523 (1971).
- ⁵ Schad, G. A., *Amer. Natur.*, **100**, 359 (1966).
- ⁶ Hussey, R. S., *J. Nematol.*, **4**, 101 (1972).
- ⁷ Ouchterlony, O., *Handbook of Immunodiffusion and Immunoelectrophoresis*, 21 (Ann Arbor Science, Ann Arbor, 1968).
- ⁸ Doubly, J. A., Flor, H. H., and Claggett, C. O., *Agric. Res. (Washington)*, **8**, 3 (1960).
- ⁹ Chester, K. S., *Q. Rev. Biol.*, **8**, 129 (1933).

- ¹⁰ Magrou, J., *C.R. Acad. Sci. (Paris)*, **3**, 257 (1935).
¹¹ Main, C. E., *Phytopathology*, **58**, 1058 (1968).
¹² DeVay, J. E., Romani, R. J., Monadjem, A. M., and Etzler, M., *Phytopathology*, **60**, 1289 (1970).
¹³ Maggenti, A. R., *Plant Parasitic Nematodes* (edit. by Zuckerman, B. M., Mai, W. F., and Rohde, R. A.), 74 (Academic Press, New York, 1971).

Remote Sensing for Productivity in Pelagic Fisheries

It has been argued that the reason for going into space is to obtain a better look at our own planet. One of the specific projects of NASA will be remote measurement of phytoplankton chlorophyll in the world's oceans, the practical use of this measurement most often mentioned relating to pelagic fisheries. A proponent is Dr Maurice Blackburn of Scripps Institution of Oceanography, who argues that the detection of phytoplankton concentrations by the measurement of chlorophyll may increase the efficiency of the tuna fisheries by reducing the scouting time necessary for the fishing fleet. Blackburn's arguments are based on a rather simple hypothesis that the distribution of a pelagic fish such as the tuna is controlled by water temperature and the availability of food¹. His group has tested this hypothesis in an area off Baja, California, where large schools of tuna concentrate, feeding primarily on a red crustacean. The crustaceans in turn feed at least partially on the phytoplankton, which are abundant in this area. There is some feeling among fishery biologists that the Baja case may be an exception. Also, if the energy change through the food web is higher than the generally accepted value of 10%, small fluctuations in primary production observed in the open ocean may be too small to explain the yield of a particular fish or its distribution². As Blackburn points out, tests with pelagic fishes are not easily arranged. The opportunity for testing the hypothesis in the Atlantic Ocean occurred when the research vessel Delaware made an exploratory

fishing cruise across the North Atlantic Ocean, roughly between the Azores and the North American coast³.

In addition to the longlining operation for tuna, standard hydrographical casts were made for temperature and salinity. Chlorophyll concentration was measured at six depths in the upper 100 m, following the method of Yentsch and Menzel⁴; total plankton volume was determined by hauling over 100 m a 0.5 m net (#6 mesh); volumes were determined using the method of Yentsch and Herbard⁵. The longline gear consisted of the Japanese type longline, with 160 fathom baskets with 87 hook design.

Largest tuna and total fish catches were made in the areas adjacent to the Azores in the North American continental shelf (Fig. 1). The big-eyed tuna dominated the catch in the eastern North Atlantic area and yellowfin in the waters of the mid-North Atlantic and Gulf Stream. Bluefin tuna were taken in the greatest numbers at the edge of the continental shelf south of Cape Cod. The big-eye tuna catch in the vicinity of the Azores reflected the catch of this fishery, which was in season at the time.

The volume of plankton and chlorophyll concentration (Fig. 1b, d) shows the same pattern of abundance as the fish (Fig. 1a, c). That is, plankton volumes and chlorophyll were highest near the eastern coast of the United States, lowest in the north central portion of the Sargasso Sea, and moderately high in the region of the Azores. The reason for the higher productivity near the eastern coast of the United States and the Azores is of interest because in both cases it is associated with ocean currents. Near the eastern coast of the United States, the dynamics of the Gulf Stream displace nutrient-rich water toward the surface and the same is true of the current moving toward the south in the region of the Azores. The magnitude of the latter is much less, and so the productivity is less.

The plankton and chlorophyll data support Blackburn's contention that in the open ocean, catches are usually greater in areas of high productivity, given that the fish are compatible with the water temperature. The data suggest that some type of aerial surveillance of chlorophyll

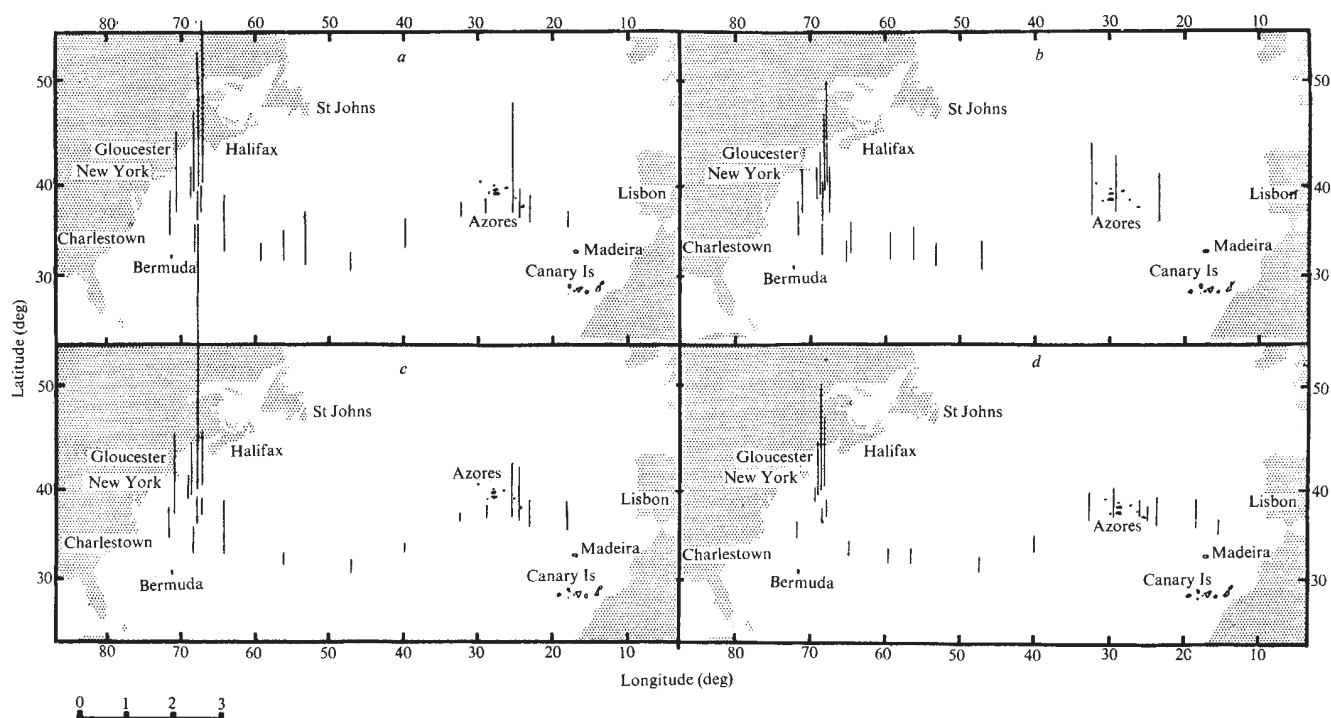


Fig. 1 Distribution of plankton and fish, April-June 1963. a, Total fish catch. One scale unit=1.0 fish per hook per hour. b, Settled plankton volume. One scale unit=10 ml. c, Tuna fish catch. One scale unit=1.0 fish per hook per hour. d, Surface chlorophyll concentration. One scale unit=0.60 $\mu\text{g l}^{-1}$.

from space would be useful. There are a number of difficulties in measuring chlorophyll from high altitudes, but recent experiments have shown that relatively simple measurements of ocean colour are an index of phytoplankton chlorophyll. It should be pointed out, however, that most of the differences of phytoplankton abundance throughout the world's oceans are the result of the same processes indicated here, that is, general oceanic circulation. Thus, global detection of water temperature alone could aid the pelagic fisheries.

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¹ Blackburn, M., *Fish. Bull.*, **68**, 147 (1969).

² Steele, J. H., *International Commission for the Northwest Atlantic Fisheries, Spec. Publ.*, **6**, 463 (1965).

³ *Cruise Report 63-4, Exploratory Fishing and Gear Research Base, Gloucester, Mass.* (US Dept. of Interior, 1963).

⁴ Yentsch, C. S., and Menzel, D. W., *Deep-Sea Res.*, **10**, 221 (1963).

⁵ Yentsch, C., and Herbard, F., *Ext. J. Cons. Int. Pour L'Exp. Mer*, **22**, 184 (1957).

Possible Discontinuous Retinal Rod Outer Segment Formation in *Latimeria chalumnae*

THE outer segments of vertebrate rods are generally agreed to consist of stacks of closed membranous disks enclosed within the outer segment membrane, unlike those of cones which are continuous with that membrane¹. In some cases the most vitread rod disks open to the ventricular space²⁻⁷. Work on the development of outer segments has suggested that the disks are formed by an infolding of the outer segment membrane in the region of the cilium¹.

Radioactive tracers have been used by Droz, Young and their colleagues to show that the proteins of rod outer segments in frogs, rats and monkeys are renewed from the vitread end. The label is first localized in the inner segment, migrates apparently through the cilium and forms a band at the vitread end of the outer segment. This band, which remains discrete, migrates

sclerally, and is eventually lost from the rod tip, when it is phagocytosed by the pigment epithelium⁷.

The inner and outer segments are connected by a modified cilium, with a 9+0 pattern of double filaments; this is usually assumed to be the sole connexion, but Tokuyasu and Yamada⁸, Pedler and Tilly⁹ and Richardson¹⁰ have demonstrated additional cytoplasmic continuity between inner and outer segments in mammals. Cohen¹ regards these examples as unusual and suggests that they may be intermittent phenomena.

During electron microscope studies on the retina of *Latimeria chalumnae* the bases of the rod outer segments have been observed to show two configurations. In about half of the rods the inner and outer segments appear clearly separated by a pair of parallel membranes. In examples where the cilium is not in the plane of section the membranes extend right across the rod (Fig. 1). Others, in which the cilium is cut, show the inner and outer segments in continuity only through that organelle, the parallel membranes separating them elsewhere.

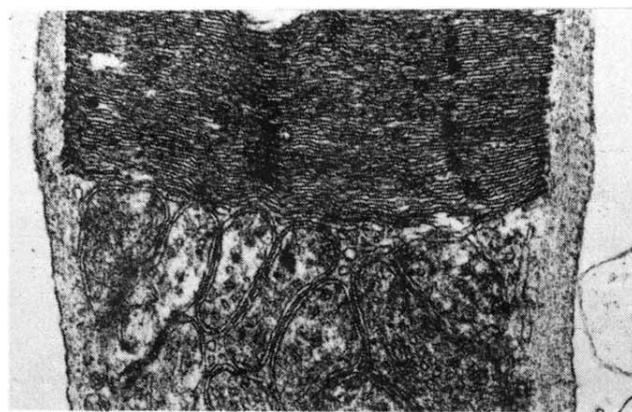


Fig. 2 In this example there is cytoplasmic continuity between the inner and outer segments, without the intervention of parallel membranes. Inner segment mitochondria are in close association with the basal outer segment lamellae, some of which are short and irregular.

Other rods do not show the paired membranes, and in these there is cytoplasmic continuity across their entire width. In these examples the inner segment mitochondria lie immediately adjacent to the outer segment base, and in places their surrounding membranes appear in series with those of the most basal lamellae. These basal lamellae usually include short ones which do not extend right across the rod (Fig. 2). In rods with this configuration and in which the cilium is cut, a lacuna is present between the shaft of the cilium and the inner segment cytoplasm. This lacuna probably represents a canal surrounding the cilium and open to the ventricular space, as described in mammals by Richardson.

These observations may be interpreted in two ways. It may be that all the rods have both an area of cytoplasmic continuity and one of separated membranes, and that the appearance of one or the other is due to accidents of the plane of section. If this is so some examples showing both features would be expected in a large sample, but none has been seen. The other possibility is that some rods showed one, and some the other, of the two configurations at the time of fixation.

If this were the case, the differences observed may not be permanent. The work of Young and his collaborators shows that outer segment protein is renewed from the vitread end as a continuous process, but that exfoliation of groups of disks from the rod tips is discontinuous. Our findings might be explained if the formation of disks is also discontinuous in *Latimeria*. The appearance in Fig. 2 would be consistent with a process of disk formation by, or in close association with, the inner segment mitochondria: that in Fig. 1 consistent with a period in which delimiting membranes had formed and the process of disk formation was in abeyance.

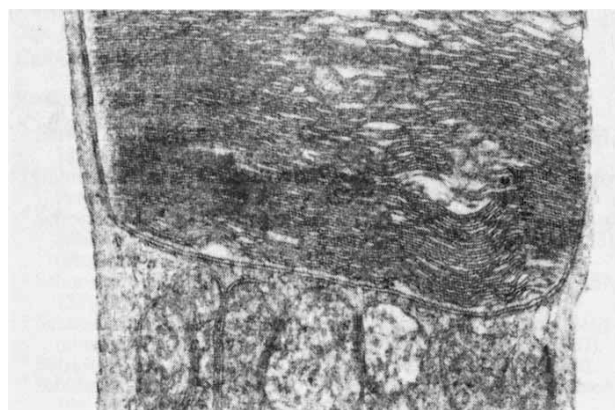


Fig. 1 Base of rod outer segment. The outer segment lamellae are enclosed within the plasma membrane, and are separated from the mitochondria of the inner segment by smooth parallel membranes. Because the cilium is not cut in this example the inner and outer segments appear to be completely separated.

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- ¹ Cohen, A. I., in *Handbook of Sensory Physiology, VIII/2, Physiology of Photoreceptor Organs* (edit. by Fuortes, M. G. F.) (Springer, New York, 1972).
- ² Moody, M. F., and Robertson, J. D., *J. biophys. biochem. Cytol.*, **7**, 87 (1960).
- ³ Cohen, A. I., in *The Structure of the Eye* (Academic Press, New York, 1961).
- ⁴ Cohen, A. I., *Exp. Eye Res.*, **2**, 88 (1963).
- ⁵ Locket, N. A., *J. mar. biol. Ass. UK*, **51**, 79 (1971).
- ⁶ Locket, N. A., *Proc. R. Soc.*, **B178**, 161 (1971).
- ⁷ Young, R. W., *J. Cell Biol.*, **49**, 303 (1971).
- ⁸ Tokuyasu, K., and Yamada, E., *J. biophys. biochem. Cytol.*, **7**, 187 (1960).
- ⁹ Pedler, C., and Tilly, R., *Exp. Eye Res.*, **4**, 370 (1965).
- ¹⁰ Richardson, T. M., *Vision Res.*, **9**, 727 (1969).

Haemodynamics in the Sauropoda

THE order Sauropoda includes the largest quadrupeds ever known. They were as long as 30 m and weighed as much as 80 t (*Brachiosaurus*¹). To maintain an adequate blood supply to the brain in animals of such enormous size², physiological adaptation is essential. If pressures similar to those of mammals are required while the animal is erect then high aortic pressures are needed to overcome the hydrostatic pressure presented by the fluid column of the carotid arteries. Special stresses are imposed on the cardiovascular system of tall animals and rapid cardiovascular adjustments are required to compensate for pressure changes occurring, for example, during foraging.

The adult giraffe, standing from 5 to 6 m (ref. 3), has a vertical profile analogous to the large sauropods. Field measurements of carotid artery pressure, taken 40 cm below the jaw, revealed pressures of 125/75 mm Hg⁴ in the quietly standing giraffe; recumbent pressures ranged up to 280/180. This study, and others^{5,6}, have substantiated that a range of pressures exists within an animal when the hydrostatic factor is relatively large. The hydrostatic factor which reduces arterial pressure in the brain increases it in the lower extremities when the body is upright.

To provide quantitative information on sauropod haemodynamics, I obtained the physical dimensions (Table 1) of two museum mounted specimens although how closely the mounted position of these specimens approximates a natural stance is difficult to assess. The position of the heart was estimated to be 46 cm below the second dorsal vertebra. Lateral measurements were taken to estimate the maximum levels to which the head could be raised.

Calculations based on the physical measurements (Table 2) ignore energy losses associated with tube dimensions and changes in blood viscosity. The values may be regarded as estimated minima if it is assumed that blood density approximated mammalian values of 1.055 g ml⁻¹ and autoregulation of cerebral vasculature was present in sauropods. Maximum hydrostatic pressure would probably occur in *Apatosaurus* only when sitting or rising upright, but not while walking; forelegs were, however, longer than

Table 1 Physical Measurements of Two Sauropods

	Distance (cm)	
	<i>Apatosaurus excelsus</i> Peabody, New Haven, Connecticut	<i>Brachiosaurus brancai</i> Humboldt, Berlin, Germany
Vertical ↓		
1 Occipital condyle to ground	475	1,187 *
2 Second dorsal vertebra to ground	320	578
3 Last cervical vertebra to ground	305	590
Lateral		
1 Occipital condyle to second dorsal vertebra	518	980 *
2 Last cervical vertebra to occipital condyle	442	878 *

* Determined by triangulation with a Brunton pocket alidade by Dr John Ostrom.

hindlegs in *Brachiosaurus* and maximum hydrostatic pressures while ambulatory would have been possible. The pressures derived from vertical distance measurements, calculated according to Burton⁷, indicate proportional changes to increasing height in a resting fluid column.

These data predict mean systemic arterial pressures of 216 mm Hg (156+60) and 568 mm Hg (508+60) for *Apatosaurus* and *Brachiosaurus* respectively, in the mounted position. These estimates assume that a pre to postcapillary pressure drop of 60 mm Hg was sufficient to allow proper brain function and that the mounted position closely approximates a normal, upright stance. If maximum vertical heights are used the pressures are increased by more than 200 mm Hg in both genera. In dependent portions of the body, systemic pressure is increased by a heart to ground hydrostatic factor and pressures of 1,000 mm Hg could have been possible.

Table 2 Calculated Vertical Displacements and Hydrostatic Pressures in Two Sauropods

	Length (cm)		Hydrostatic pressure (mm Hg)	
	Mounted	Maximum	Mounted	Maximum
<i>Apatosaurus</i>				
Heart-brain	201	473	156	367
Heart-ground	274	—	213	—
<i>Brachiosaurus</i>				
Heart-brain	655	924	508	717
Heart-ground	532	—	413	—

Clearly the cardiovascular pressures in sauropods could have been enormous and this suggests that an appraisal of the assumptions used in estimating the pressures might be helpful. Is 60 mm Hg a realistic cerebral perfusion pressure? What evidence supports cerebral autoregulation or cardiovascular regulation in reptiles at any level? Figure 1 shows femoral arterial pressure in an anaesthetized iguana (*Iguana iguana*). This record was obtained at 28° C by cannulating the femoral artery with a saline-filled polyethylene catheter connected to a Statham 23A pressure transducer and recorded on a Grass model 7 polygraph. The record illustrates that systemic pressures approaching those of mammals are characteristic of at least some present-day reptiles. In addition, Hohnke (in preparation) has demonstrated that in the conscious iguana, arterial blood pressure is regulated during periods of haemorrhage and passive uptilting of the head. This indicates controlled pressure in the cerebral vasculature and provides evidence of cardiovascular compensation mechanisms in reptiles generally.

Accepting the high arterial blood pressures predicted by the calculations, it is interesting to speculate on structural

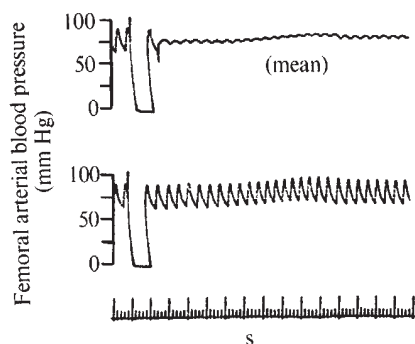


Fig. 1 Arterial blood pressure in *Iguana iguana*.

adaptations of the cardiovascular system. The law of Laplace has important implications for the structure of the ventricle when such high pressures are necessary. A small ventricular radius with thick myocardium would favour the maintenance of the required high cardiac pressures. Blood vessel structure was probably not too different from that of the giraffe. Arteries in the giraffe, such as the metatarsal, have large wall:lumen ratios³. Arteries closer to the head, carrying lower pressures, have lower ratios. Similar morphological changes accompany the development of essential hypertension in man⁸.

Peripheral vascular beds such as muscle and kidney probably showed specialized adaptations also. In muscle, high capillary pressures could cause oedema unless counterbalanced by high plasma oncotic pressures. High filtration pressures in the kidney could enhance urine formation and require a considerable amount of energy for reabsorption of water and essential electrolytes. Precapillary attenuation of high arterial pressures would have been desirable and economical in both vascular beds.

The success of large reptiles during the Jurassic and Cretaceous periods proves that these animals solved the problem associated with great distances between the heart and the brain. Obviously, the exact cardiovascular values and specific adaptations of sauropods will never be known and inferences concerning their physiology must rest heavily upon assumptions. These calculations, based on skeletal measurements, have quantified in a limited way the haemodynamics of two sauropods and predict very high pressures in the aorta, and imply reflexogenic control over cerebral perfusion pressures.

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- ¹ Colbert, E. H., *Dinosaurs: Their Discovery and Their World* (Dutton, New York, 1961).
- ² Bakker, R. T., *Nature*, **229**, 172 (1971).
- ³ Goetz, R. H., and Keen, E. N., *Angiology*, **8**, 542 (1957).
- ⁴ Van Citters, R. L., Kemper, W. S., and Franklin, D. L., *Science*, **152**, 384 (1966).
- ⁵ Patterson, J. L., Goetz, R. H., Doyle, J. T., Warren, J. V., Gauer, O. H., Detweiler, D. K., Said, S. I., Hoernicke, H., McGregor, M., Keen, E. N., Smith, M. H., Hardie, E. L., Reynolds, M., Flatt, W. P., and Waldo, D. R., *Ann. NY Acad. Sci.*, **127**, 393 (1965).
- ⁶ Goetz, R. H., Warren, J. V., Gauer, O. H., Patterson, J. L., Doyle, J. T., Keen, E. N., and McGregor, M., *Circulation Res.*, **8**, 1049 (1960).
- ⁷ Burton, A. C., *Physiology and Biophysics* (edit. by Ruch, T. C., and Patton, H. D., Philadelphia, 1965).
- ⁸ Sivertson, R., *Acta Physiol. Scand.*, **79**, Suppl. 343 (1970).

Competition and Diversity in Herbaceous Vegetation

GRIME¹ points out that herbaceous vegetation on highly favourable, "low-stress" sites often contains few species compared with that on intermediate-stress sites. He proposes an explanation for this which rests upon the claim that competition is less intense at intermediate-stress sites than at low-stress sites. I question this claim. The only evidence Grime gives to support it is based on a competitive index which he has devised. But he uses characters which are mainly related to competition for light, and seems to ignore the possibility of root competition. That root competition does occur between grassland plants is shown by experiments in which either shoot or root competition was prevented by partitions between the plants²⁻⁷. (Admittedly the conditions in such experiments are artificial.) Evidence that competition can be intense in species-rich grassland is as follows. (1) If seeds are sown into such vegetation and into control plots from which the vegetation has been removed, seedling establishment and growth are often better in the control plots^{8,9}. (2) If some species are removed from established vegetation, some (at least) of the remaining species show increased growth^{9,10}. (3) If organic nitrogenous fertilizer is added to the soil, the bulk of vegetation usually increases¹¹⁻¹⁴. This shows that at such sites nitrogen is usually a factor limiting growth, and as nitrate and ammonium ions are mobile in soil, it is virtually certain that before addition of the fertilizer the plants were competing for nitrogen.

In my view, Grime's hypothesis should be altered, to state that when competition for light is intense, species diversity tends to be low; when competition for light is reduced, whether because of frequent cutting, trampling, low nutrient supply leading to intense root competition, or for other reasons, species diversity is often higher. I can suggest two possible reasons why competition for light, but not root competition, leads to low diversity. First, when plants are competing for light, a slight difference in tallness can give one plant a significant advantage, which with time "snowballs" until the shorter plant is totally suppressed¹⁵. In root competition it is doubtful whether any single character would confer such an advantage on one species. Second, when growth is limited mainly by light, there is little opportunity for small-scale habitat diversity, which would provide ecological niches. In contrast, in the soil there can be many niches; for example, plants can root at different depths, they can respond to small-scale variations in soil properties¹⁶, and their roots can occupy different-sized soil pores¹⁷.

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- ¹ Grime, J. P., *Nature*, **242**, 344 (1973).
- ² Donald, C. M., *Aust. J. agric. Res.*, **9**, 421 (1958).
- ³ Wilkinson, S. R., and Gross, C. F., *Agron. J.*, **56**, 389 (1964).
- ⁴ Bland, B. F., *J. agric. Sci. (Camb.)*, **69**, 391 (1967).
- ⁵ King, J., *J. Br. Grassld. Soc.*, **26**, 221 (1971).
- ⁶ Snaydon, R. W., *J. appl. Ecol.*, **8**, 687 (1971).
- ⁷ Eagles, C. F., *J. appl. Ecol.*, **9**, 141 (1972).
- ⁸ Rorison, I. H., *J. Ecol.*, **48**, 585 (1960).
- ⁹ Sagar, G. R., and Harper, J. L., *Weed Res.*, **1**, 163 (1961).
- ¹⁰ Putwain, P. D., and Harper, J. L., *J. Ecol.*, **58**, 251 (1970).
- ¹¹ Brechley, W. E., and Warington, K., *The Park Grass Plots at Rothamsted* (Rothamsted Experimental Station, Harpenden, 1958).
- ¹² Willis, A. J., *J. Ecol.*, **51**, 353 (1963).
- ¹³ Lloyd, P. S., and Pigott, C. D., *J. Ecol.*, **55**, 137 (1967).
- ¹⁴ Smith, C. J., Elston, J., and Bunting, A. H., *J. Br. Grassld. Soc.*, **26**, 213 (1971).
- ¹⁵ Black, J. N., *Aust. J. agric. Res.*, **9**, 299 (1958).
- ¹⁶ Snaydon, R. W., *J. Ecol.*, **50**, 133 (1962).
- ¹⁷ Sheikh, K. H., and Rutter, A. J., *J. Ecol.*, **57**, 713 (1969).

DR GRIME replies: There is a clear difference of opinion between Newman and myself with regard to the role of root competition in natural vegetation. It would be a mistake to pursue the discussion, however, without first defining competition and placing the phenomenon in a wider but relevant context.

Following the arguments (but not the wording) of Milne¹, competition between plants may be defined as the tendency of neighbouring plants to utilize the same quanta of light, ions of mineral nutrients, molecules of water or volume of space. This definition recognizes that competition is merely one component of Darwin's² struggle for existence and that in many habitats effects of competition are less important than those arising from differential susceptibility to the direct impact of the physiochemical environment or to damage by man and other predators. The need to observe these distinctions in the present discussion is obvious if we recognize that competition has led to the evolution of strategies which are different from those which enable plants to withstand extremes of environmental stress or predation.

I contend that the intensity of competition, above and below ground, reaches a maximum in fertile habitats where there is little damage by predators. The terminal role of shading in competition on fertile soils should not be allowed to obscure the fact that in such circumstances competition for nutrients is severe and may be of critical importance. An illustration of this is provided by the experiment of Donald³ in which the superior competitive ability of *Lolium perenne* over *Phalaris tuberosa*, in conditions of high fertility, is pronounced only in treatments which permit root competition.

The weakness in Newman's argument is the assumption implicit in the phrase "low nutrient supply leading to intense root competition". Whilst it is clear that on nutrient deficient soils root competition is more important than competition for light, there are no grounds for supposing that the intensity exceeds that which occurs on fertile soils. From an examination of the relevant literature⁴⁻⁸, I support the conclusion of Higgs and James⁷ that there is no convincing evidence that plants indigenous to poor soils are more efficient in the uptake of essential nutrients. Moreover, field observations and experiments⁹⁻¹¹ strongly suggest that the ability of these inherently slow-growing⁸ plants to survive in infertile habitats is related to their ability to persist for long periods in a state of chronic mineral nutrient deficiency and in this condition to resist the impact of climatic stress and the attentions of predators.

I would suggest, therefore, that the high species densities often encountered on unproductive soils are the result of the low competition in general rather than the shift in relative importance from shoot to root competition.

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Received May 31, 1973.

¹ Milne, A., in *Mechanisms in Biological Competition* (edit. by Milnthorpe, F. L.), 40 (Cambridge University Press, London, 1961).

² Darwin, C., *The Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life* (Murray, London, 1859).

³ Donald, C. M., *Aust. J. agric. Res.*, **9**, 421 (1958).

⁴ Bradshaw, A. D., Chadwick, M. J., Jowett, D., and Snaydon, R. W., *J. Ecol.*, **52**, 665 (1964).

⁵ Hackett, C., *J. Ecol.*, **53**, 315 (1965).

⁶ Clarkson, D. T., *J. Ecol.*, **55**, 111 (1967).

⁷ Higgs, D. E. B., and James, D. B., *J. Ecol.*, **57**, 553 (1969).

⁸ Hunt, R., thesis, Univ. Sheffield (1970).

⁹ Kruckeberg, A. R., *Ecology*, **35**, 267 (1954).

¹⁰ Grime, J. P., *J. Ecol.*, **51**, 391 (1963).

¹¹ Davison, A. W., thesis, Univ. Sheffield (1964).

Corrected Chromosome Number for *Spartina* in Ireland

ON the basis of the chromosome count of $2n=126\pm 2$ (ref. 1), *Spartina townsendii* H. & J. Groves was generally considered to be $2n=126$ until 1963, when Marchant (ref. 2) first recorded $2n=120$, 122 and 124 for plants in Britain. Seed-producing *S. townsendii* at Baldoyle near Dublin has also been found to have $2n=124$ (ref. 3). A preliminary account of this grass was given in 1961 (ref. 4). The account is correct in general essence (ref. 3) but the chromosome number was incorrectly stated to be $2n=126$ and a photograph used in illustration was misinterpreted as showing three quadrivalents and fifty-seven bivalents. In fact, the photograph shows one trivalent, one univalent and either two quadrivalents and fifty-six bivalents or sixty bivalents.

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¹ Huskins, C. L., *Genetica*, **12**, 531 (1930).

² Marchant, C. J., *Nature*, **199**, 929 (1963).

³ Boyle, P. J., thesis, University College, Dublin (1972).

⁴ Boyle, P. J., and Kavanagh, J. A., *Nature*, **192**, 81 (1961).

Geological Antiquity of Rhodesian Man

CARLETON COON, in his book, *The Origin of Races*¹, argued that the "Caucasoid" (White) race, as represented by the well-known Swanscombe and Steinheim skulls, had crossed the threshold from *Homo erectus* to *Homo sapiens* in the Middle Pleistocene, at least by 250,000 yr ago, whereas the "Congoid" (Negro) race, as represented by the massive-browed and low-vaulted Broken Hill and Saldanha skulls, had remained at the *Homo erectus* level until well within the Upper Pleistocene, perhaps as recently as 30-40,000 yr ago. It has been pointed out that there are grounds for questioning the assignment of the Broken Hill and Saldanha specimens (lumped here and elsewhere as Rhodesian man) to *Homo erectus*² and few authors would agree that they can be assigned to any modern race, but most have accepted their Upper Pleistocene date. In this note I point out recent evidence that these specimens are in fact far older.

The principal Broken Hill specimen, nearly complete except for the mandible, was found by lead miners in a pocket of animal bones exposed in 1921 in a cave near Broken Hill, Northern Rhodesia (now Kabwe, Zambia)³. A minimum of twenty-three large mammal species were represented⁴, including at least five extinct forms—a large baboon (?*Simopithecus*), a sabretooth cat (machairodont), a small warthog (?*Tapinochoerus*), a short-necked giraffid (cf. *Libytherium*), and a giant buffalo ("*Homoioceras*" = *Pelorovis*). A small and incomplete collection of artefacts that were probably associated with the skull was initially assigned by Clark^{5,6} to the Proto-Stillbay industry (=Charaman of Cooke^{6,7}) and more recently to the Sangoan⁸, a terminal Early Stone Age ("First Intermediate") industry.

The more fragmentary Saldanha skull, which consists of a calvarium and a mandibular ramus fragment, was found in 1953 by Jolly and Singer on the surface at Elandsfontein (Hopefield), about 130 km north of Cape Town and 24 km east of Saldanha Bay, South Africa⁹⁻¹¹. The faunal associations of the Saldanha skull are not entirely clear, because, like the skull, most of the faunal material has been collected from the surface, but it is

probable that the hominid and the bulk of the mammalian taxa were roughly contemporaneous, as is suggested by Oakley's geochemical analyses of skull and animal bones¹². Approximately fifty large mammal species have been identified¹³, of which no less than nineteen are extinct, including most notably a giant baboon (*Simopithecus*), a sabretooth (*Megantereon*), a pig (*Mesocherus*), a warthog (*Tapinochoerus*), a short-necked giraffid (*Libytherium*), a giant hippotragine antelope (*"Hippotragus" gigas*), a giant buffalo (*Pelorovis*), and various alcelaphine antelopes (especially *Megalotragus*, *Rabaticeras*, and *?Beatragus*). The artefactual associations of the hominid skull are difficult to establish, but it has generally been assumed that Saldanha man made at least some of the Acheulean (Early Stone Age) handaxes found at the site¹⁴.

The radiocarbon dates that were available in the late 1950s and 1960s suggested that the Early Stone Age (s.l)/Middle Stone Age interface lay somewhere within the Upper Pleistocene, probably about 40,000 BP^{8,15-17}, and therefore most workers, including Coon, have assumed that the Broken Hill skull was roughly 40,000 yr old or even somewhat younger. The Saldanha skull has been considered older, but not necessarily very much so, because the handaxes with which it may have been associated were assigned to an "evolved" Acheulean (the terminal Early Stone Age or "First Intermediate" Fauresmith industry¹⁴). The archaic nature of the associated faunas, especially of the richer and better known assemblage from Elandsfontein, has been assumed to reflect the very late survival of quite archaic forms in sub-Saharan Africa.

Recent re-evaluation of the radiocarbon chronology of the southern African Stone Age by Vogel and Beaumont^{18,19}, based on an important series of fresh dates, clearly suggests that the Middle Stone Age may have ended, rather than begun, at 30-40,000 BP, and the Early Stone Age (s.l)/Middle Stone Age interface probably lies beyond the limits of the carbon-14 technique. Independent geological evidence for the unexpectedly great antiquity of the Middle Stone Age has been developed at the Klasies River Mouth and Nelson Bay caves on the southern Cape coast (South Africa), where long Middle Stone Age successions begin on and even in raised beaches probably dating from the Last (Eem) Interglacial²⁰⁻²². By implication, the Early Stone Age (s.l)/Middle Stone Age interface may therefore lie in the Middle Pleistocene (that is, predate the Last Interglacial), and it is not even inconceivable that some so-called Middle Stone Age industries may date from well within that interval.

Recent analysis of the thousands of identifiable mammal bones excavated from the Middle Stone Age layers of the Klasies River Mouth caves suggests that the fauna, with a minimum of forty-three large mammal species, was essentially (generically) modern²³. Only two extinct genera—*Pelorovis* and *Megalotragus*—were represented (the latter not certainly), and it is now known that both genera persisted until the very end of the Pleistocene (10-12,000 BP) in South Africa²³. If the terminology of Cooke²⁴ and Wells²⁵ is followed and the essentially modern fauna of Klasies is assigned to the "Florisbad-Vlakkraal faunal span", then the implication is that the interface with the preceding "Vaal-Cornelia span" including the Elandsfontein and Broken Hill faunas must fall within the earliest Upper Pleistocene (Eem Interglacial) or Middle Pleistocene. Indeed an estimate of how old the Elandsfontein and Broken Hill faunas may be can be obtained from their broad similarity to that of Olduvai Upper Bed II through Bed IV^{26,27}. Firm dates for these Olduvai units have not yet been published, but even the youngest (Bed IV) has been tentatively bracketed between 700,000 and 400-300,000 BP by a combination of palaeomagnetic evidence and extrapolated sedimentation rates²⁸.

In sum, the conventional Upper Pleistocene or even

later Upper Pleistocene dating of the Saldanha and Broken Hill skulls, on which Coon partly based his argument, is almost certainly incorrect. The precise age(s) of the two skulls remains uncertain, but a combination of artefactual and faunal evidence suggests that they date from the later part of the Middle Pleistocene, more than 125,000 yr ago, if the best current estimate for the beginning of the Last Interglacial²⁹ is accepted as the end of the Middle Pleistocene. It is quite possible, perhaps even probable, that they are significantly older than the undoubted *Homo sapiens* skulls found in the Kibish Formation of southern Ethiopia and tentatively dated by Th/U to 130,000 BP^{30,31}. Whether they are best regarded on morphological grounds as late *Homo erectus*^{1,32} or archaic *Homo sapiens*³³⁻³⁵, it is clear that they are much further back on the *Homo erectus*-*Homo sapiens* continuum than Coon thought, and certainly do not constitute good evidence for a very late persistence of *Homo erectus* in sub-Saharan Africa.

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Received March 5; revised May 7, 1973.

- ¹ Coon, C. S., *The Origin of Races* (Knopf, New York, 1962, 1966).
- ² Howells, W. W., *Sci. Am.*, **215**, 46 (1966).
- ³ Clark, J. D., Oakley, K. P., Wells, L. H., and McClelland, J. A. C., *J.R. anthrop. Inst.*, **77**, 7 (1950).
- ⁴ Leakey, L. S. B., *J.R. anthrop. Inst.*, **89**, 225 (1959).
- ⁵ Clark, J. D., *J.R. anthrop. Inst.*, **89**, 201 (1959).
- ⁶ Cooke, C. K., *Arnoldia*, **2**, 22 (1966).
- ⁷ Cooke, C. K., *Arnoldia*, **3**, 39 (1968).
- ⁸ Clark, J. D., *The Prehistory of Africa* (Thames and Hudson, London, 1970).
- ⁹ Drennan, M. R., *Nature*, **172**, 791 (1953).
- ¹⁰ Singer, R., *Am. J. phys. Anthrop.*, **12**, 345 (1954).
- ¹¹ Drennan, M. R., and Singer, R., *Nature*, **175**, 364 (1955).
- ¹² Oakley, K. P., *Proc. Third pan-Afr. Congr. Prehist.* (edit. by Clark, J. D.), 76 (Chatto and Windus, London, 1957).
- ¹³ Hendey, Q. B., *Ann. S. Afr. Mus.* (in the press).
- ¹⁴ Singer, R., and Crawford, J. R., *J.R. anthrop. Inst.*, **88**, 11 (1958).
- ¹⁵ Clark, J. D., *The Prehistory of Southern Africa* (Penguin, Harmondsworth, 1959).
- ¹⁶ Clark, J. D., *Science*, **150**, 833 (1965).
- ¹⁷ Klein, R. G., *S. Afr. archaeol. Bull.*, **25**, 127 (1970).
- ¹⁸ Vogel, J. C., and Beaumont, P. B., *Nature*, **237**, 50 (1972).
- ¹⁹ Beaumont, P. B., and Vogel, J. C., *Afr. Stud.*, **31**, 65 (1972) and **31**, 155 (1972).
- ²⁰ Wymer, J. J., and Singer, R., *Man, Settlement and Urbanism* (edit. by Ucko, P. J., Tringham, R., and Dimbleby, G. W.), 207 (Duckworth, London, 1972).
- ²¹ Butzer, K. W., *S. Afr. archaeol. Bull.*, **28** (in the press).
- ²² Klein, R. G., *Wild Archaeol.*, **5** (in the press).
- ²³ Klein, R. G., *Quat. Res.*, **2**, 135 (1972).
- ²⁴ Cooke, H. B. S., *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.), 175 (University of Chicago Press, Chicago, 1967).
- ²⁵ Wells, L. H., *S. Afr. archaeol. Bull.*, **95** and **96**, 93 (1969).
- ²⁶ Leakey, L. S. B., *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.), 7 (University of Chicago Press, Chicago, 1967).
- ²⁷ Leakey, M. D., *Olduvai Gorge*, **3** (Cambridge University Press, Cambridge, 1971).
- ²⁸ Leakey, M. D., *Nature*, **232**, 380 (1971).
- ²⁹ Butzer, K. W., *Environment and Archaeology* (rev. edit.) (Aldine-Atherton, Chicago, 1971).
- ³⁰ Leakey, R. E. F., Butzer, K. W., and Day, M. H., *Nature*, **222**, 1137 (1969).
- ³¹ Butzer, K. W., Brown, F. H., and Thurber, D. L., *Quaternaria*, **11**, 15 (1969).
- ³² Arambourg, C., *Archs Inst. Paléont. hum.*, mém. **32**, 37 (1963).
- ³³ Wells, L. H., *Proc. Third pan-Afr. Congr. Prehist.* (edit. by Clark, J. D.), 172 (Chatto and Windus, London, 1957).
- ³⁴ Howell, F. C., and the editors of Time-Life Books, *Early Man* (Time-Life Books, New York, 1965, 1970).
- ³⁵ Pilbeam, D. R., *The Ascent of Man* (Macmillan, New York, 1972).

Reconstruction of the Dental Arcades of *Ramapithecus wickeri*

SPECIES of *Ramapithecus* are among the few hominoid species currently considered as possibly close to the direct line of human ancestry. One of the most striking resemblances between *Ramapithecus* and later hominids is the supposed presence of parabolic dental arcades¹. In view of the important evolutionary position postulated for these imperfectly known species we have made a reconstruction of the mandible and lower part of the face of the Kenyan species *R. wickeri* (Leakey)^{2,3}. Previous attempts at reconstruction⁴⁻⁶ have been necessarily conjectural because there were no midline points upon which tooth row divergence could be based. The mandible of *R. wickeri* (KNM-FT 45)³ is so far the only specimen of this species, or of the closely related *R. punjabicus* (Pilgrim), that has the midline preserved at any point. It is also likely that it is associated with the maxilla specimen (KNM-FT 46).

A new examination of the left mandibular fragment shows that much of the symphysis is preserved. It now seems that the alveolus identified previously as that of the central incisor is actually that of the right lateral incisor and, further, that part of the medial wall of the right canine alveolus is also seen below it on the fractured surface. The anterior fracture across the symphysis is so low down that only the very deepest parts of the central incisor alveoli are seen on either side of the midline. The left lateral incisor root socket is preserved intact and was considerably larger than that of the central incisor; and the right lateral incisor socket is cut across longitudinally by the break in the mandible. The bony features of the symphysis are seen in the section in anterior view (Fig. 1) and a very low angulation is found to mark the midline on the anteroinferior symphyseal surface.

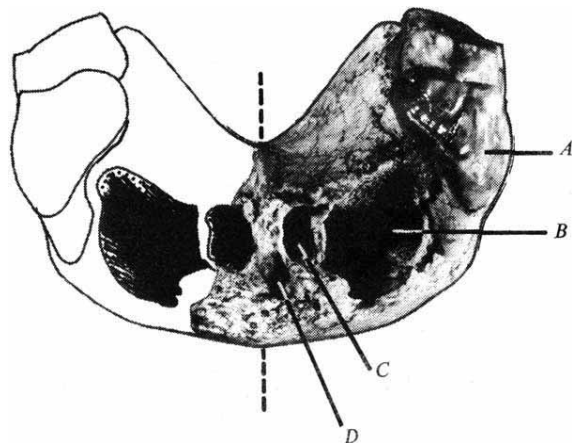


Fig. 1 Anterosuperior view of mandibular fragment, showing midline (dotted line). Missing parts of the right side are sketched in. A, P_3 crown; B, left canine alveolus; C, left lateral incisor alveolus; D, deepest part of left central incisor alveolus.

To make the reconstruction, mirror image replicas were modelled in plaster and joined to plaster casts of the original specimen. The mandibular antimeres could be accurately joined along the plane of the symphysis, as indicated by the dotted line in Fig. 1. To complete the reconstruction of the anterior part of the mandible the missing parts of the incisive alveolar margins were filled in following the contours of the surrounding good bone and an indication of the incisor crowns was modelled. The canines are based on a recently discovered right canine (KNM-FT 3318) and its mirror image. The posterior part of the reconstructed mandible is based partly on another undescribed specimen (KNM-FT 7), a right mandibular fragment with the crowns of P_4 and M_1 and the buccal margin of the body below P_4 - M_2 preserved. Photographs

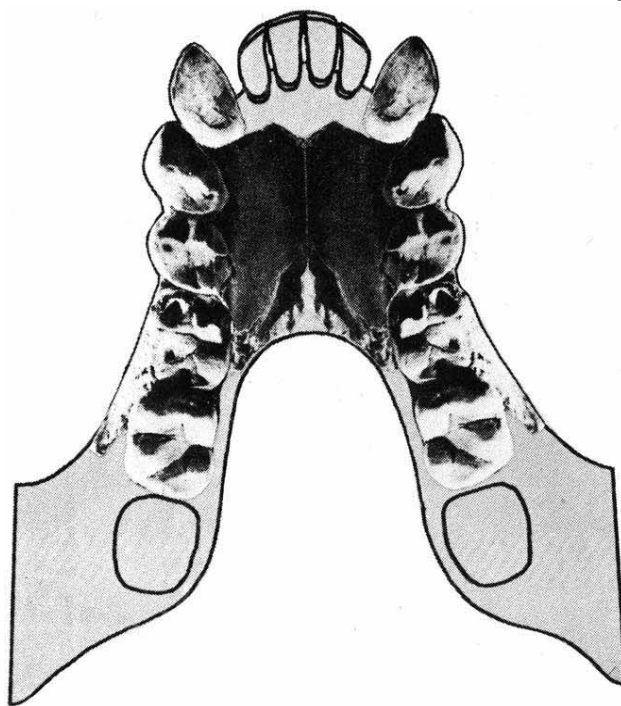


Fig. 2 Occlusal view of reconstructed mandible.

and reversed photographs are presented rather than photographs of the model in order more clearly to show detail and the reconstructed areas.

The mandibular dental arcade is shown by the reconstruction to be very narrow anteriorly and the cheek-tooth rows to diverge posteriorly (Fig. 2). The anterolateral angle of the mandibular body is dominated by the jugum for the mesial root of P_3 . In pongids the canine jugum marks the anterolateral angle of the body, but in *R. wickeri* the canine is mesiolingually placed with respect to P_3 and the long axes of these two teeth overlap each other over more than half their lengths. Similarly the incisors are mesiolingually emplaced with respect to the canine with the result that the anterior border of the mandible is abbreviated and the lingual border of the incisive alveolar margin is on a line that runs just mesial to the third premolars. The reconstruction emphasizes the great posterior development of the inferior mandibular torus which reaches as far as the level of M_1 .

The left and right maxillary fragments (KNM-FT 46 and 47) were used for the maxillary reconstruction. These have parts not common to both sides and these parts were modelled on to the plaster casts of their opposites. This meant modelling, essentially, the canine and premolar region on the right fragment and the maxillary tuberosity and base of crown of the M^3 on the left fragment. The two completed parts were then placed in the best-fit centric occlusal position on the mandibular model and united in that position. The approximate size and shape of the M^3 crowns were judged from the crown outline present on the right maxillary fragment. The premaxillary region was modelled as a functional counterpart of the anterior end of the mandibular model. This reconstruction is shown in Figs 3 and 4.

As in the mandible, the maxillary tooth rows are shown by the reconstruction to be anteriorly approximated and to diverge posteriorly (Fig. 3). The palate appears relatively long and narrow and an estimated index (internal width between the first molars/ C - M^3 tooth row length) is 40%. This compares well with values for the gorilla (35-50%), is lower than for the chimpanzee (54-71%) and is much lower than values for modern man. Alveolar prognathism is slight and the premaxillary region is quite abbreviated. As judged from the remaining portions of the zygomatic processes, the face was extremely flat and broad posterior to the canine fossa. A full face view of the

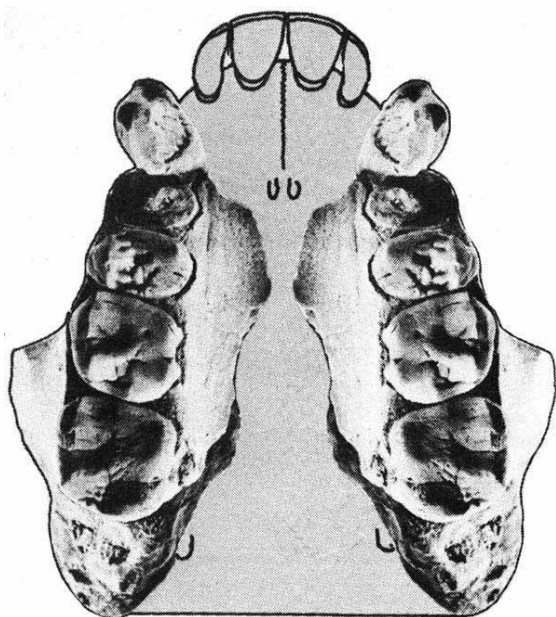


Fig. 3 Occlusal view of reconstructed palate.

reconstruction was attempted but is not presented because the shading of the missing portions obscures the photographs almost entirely.

The outlined shaded portions of the reconstruction in Fig. 4 are based on filling in between known points or on regressions based on known measurements. Regressions between variables were based on a sample of forty-eight modern primate skulls (eleven *H. sapiens*, twenty-five pongids and twelve cercopithecoids). A regression of nasopinale to alveolare on zygomatic height (cervical border of M^1 to highest point on the zygomatic process of the maxilla) gave a mean nasospinale-alveolare distance of 16.0 against a measured zygomatic height of 13.1. This

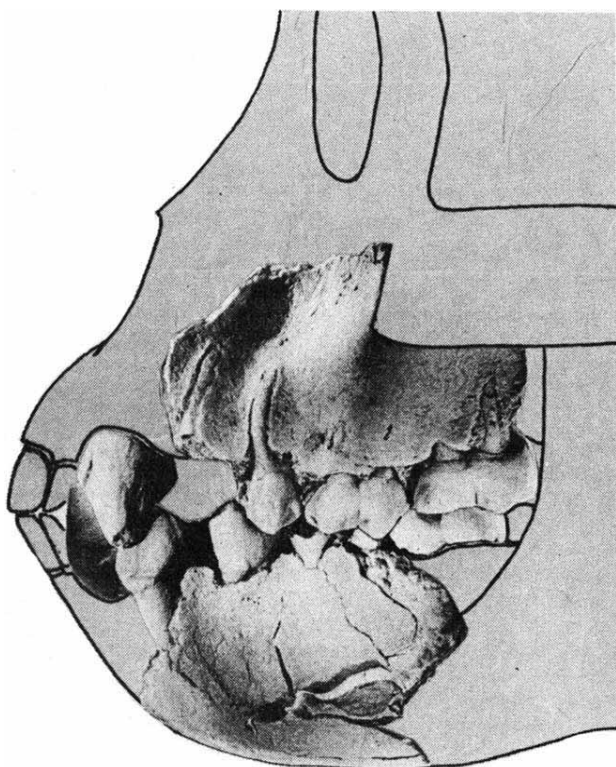


Fig. 4 Lateral view of reconstructed mandible and maxilla.

estimated value is the same as that measured on the original *R. punjabicus* maxilla YPM 13799. Another facial estimate was obtained from the regression of the minimum distance from the floor of the orbit to the cervical border of P^4 on zygomatic height, which gave a mean value of 29.6.

Several points arise from this reconstruction. It seems likely that *R. wickeri* had very small incisors indeed and posteriorly diverging and nearly straight cheek-tooth rows, rather than widely separated and curved tooth rows such as are found in modern man. The palate is narrow and the tooth rows are relatively very elongated. The premaxillary region was most likely abbreviated and a small snout with a small piriform aperture projected from a broad and very flat face that was especially wide in the zygomatic region. These peculiar and unique gnathic features very clearly place *Ramapithecus wickeri* apart from contemporary species of *Dryopithecus* and also make it clear that it did not have the rounded dental arcade postulated from previous reconstructions.

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¹ Simons, E. L., and Pilbeam, D. R., *Folia Primat.*, **3**, 81 (1965).

² Leakey, L. S. B., *Ann. Mag. Nat. Hist.*, **4**, 689 (1962).

³ Andrews, P., *Nature*, **231**, 192 (1971).

⁴ Simons, E. L., *Postilla*, **57**, 1 (1961).

⁵ Pilbeam, D. R., *Bull. Peabody Mus. Nat. Hist.*, **31**, 1 (1969).

⁶ Genet-Varcin, E., *La Recherche du Primate Ancêtre de l'Homme* (Boubee, Paris, 1969).

⁷ Trevor, J. C., *Anthropometry in Chambers's Encyclopaedia*, 458 (Newnes, London, 1950).

Reasoning and Piaget

THE recently published article by Bryant and Trabasso¹ gives the impression that something significantly new about children's inference has been discovered which, in turn, challenges Piaget's² analysis of operative development. The thesis and the data on which it is based deserve critical commentary because the first claim is questionable and the latter is incorrect.

The authors assert that they were able to measure "genuine transitive inferences" in children as young as 4 yr because they eliminated memory as a factor whereas other investigators did not. Children were trained and overtrained to discriminate sequential pairs of rods AB, BC, CD, DE; the rods differed in colour and children had to label each rod with a name, big or small. The critical test was nonsequential pairings of the rods BD, success on which was said to be an inference based on memory for the old pairs: the probability that rod B is called longer than rod D is jointly determined by probabilities of recalling that B was bigger than C and that C was bigger than D.

With the exception of comparing the obtained with the expected success on new pairs, no experimental manipulation of the memory factor was made and neither frequency of pairings nor time was varied. The authors did not manipulate the pre-inference sequence, a condition which is known to implicate memory on this kind of problem³ and their interpretation of memory is, therefore, far from convincing.

In the light of established literature on this problem, the absence of controls which might have made the data clearer is inexcusable. To show inference, investigators have usually asked children to judge pairs of rods in conditions of varying amounts of information. Control conditions included: (1) showing BD without other prior comparisons; (2) showing BD after pairing each with other rods, but not with a middle term⁴; (3) varying transitivity itself by having the same subjects judge BD after premises $B > C = D$, $B = C > D$, and $B > C > D^{3-5}$; (4) having BD judged in comparative conditions when logical and perceptual information would be in conflict⁶; and (5) neutralizing possible designations of B and D in absolute size terms. Bryant and Trabasso¹, who used only the last control and found no age differences, cannot claim that they discovered a reasoning capacity apart from memory unless they found subjects who successfully learnt the original paired discriminations and failed on the BD pairing. Thus in their design, memory, learning, attention, and inference are inextricably confounded and cannot be related to a developmental framework.

Apart from irregularities of method we question the naive assumption of the authors that any size discrimination not based on a direct perceptual encounter is *ipso facto* logical reasoning. Usually in an inference, a necessary, logical conclusion is drawn from a set of premises. But here we have no more than a perceptual consistency; children were working with arbitrary relations among differently coloured rods. To be parsimonious, their subjects can be said to have performed consistently in judging new pairs in an organized system dictated by the old, overlearned pairs.

This may be a type of inference arrived at by perceptual generalization or consistency but it is obviously different from logical inference studied by Piaget. Other investigators^{7,8} have also studied "sub-logical" inferences and found that very young children are capable of consistency in responding to new items through relations entailed by old. Huttenlocher⁹ has shown that persons can reason according to relations in an imaginal manner through which the common link is reinstated. For example, when Charles is bigger than Mary, and Mary is bigger than Bill, the child imaginably orders Charles > Mary > Bill and concludes that Charles is bigger than Bill. It has also been shown that children can order colours in this same way^{7,8}; the requirement in the task of Bryant and Trabasso¹.

More important than the methodological weaknesses and alternative explanations of the data is the criticism levelled at Piaget's theory. A rereading of Piaget's work would show that the situations in which Piaget has studied transitive inference do not entail memory processes. For example, the child stands between two towers of blocks, each on a table of differing height, and is asked to find out whether the two towers are the same or different in height. Piaget observed that children below ages of 6 or 7 yr guessed, used visual estimation, or improvised imprecise measuring tools such as an arm span. Older subjects used logical strategies for obtaining a certain answer. They made use of material with which to measure the comparative heights so that the measuring instrument became a logical middle term for example, $A > B$, $B = C$, therefore $A > C$.

It does not seem reasonable to claim that Piaget's observations of an age separation are explained by a memory factor. Younger children did not use a measuring device, or when they did so its use was unreliable. Older children used devices which allowed for sure judgments. For all children the towers and potentially usable middle terms were always present on the tables in front of the children; consequently there was no information to remember or forget.

In fact, Bryant and Trabasso's work is not germane to Piaget's observations; it refers to studies of other investigators who have used the method of successive discriminations. But, as we said before, memory has already been found to play a role in this kind of study and with adequate controls Piaget's developmental model has been supported.

Perhaps a broader issue is involved here. Today, Piaget's theory seems fair game to almost any type of reductive analysis

based on elementary processes like memory, attention, learning, and so on. It is not clear where these several polemic arguments are leading us. On the one hand, they attempt to discredit the theory even though Piaget has recognized the importance of these processes in operative development¹⁰. On the other hand, they offer little in terms of constructive alternatives. A broader perspective like that taken by ethologists¹¹ would be more helpful in the task of uncovering the relation of learning to development. Fortunately for students of human development, Piaget's theory has already provided a foundation from which we can build.

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- ¹ Bryant, P. E., and Trabasso, T., *Nature*, **232**, 456 (1971).
- ² Piaget, J., Inhelder, B., and Szeminska, A., *The Child's Conception of Geometry* (Routledge and Kegan Paul, London, 1960).
- ³ Youniss, J., and Murray, J. P., *Devl. Psychol.*, **2**, 169 (1970).
- ⁴ Youniss, J., and Dennison, A., *Child Dev.*, **42**, 1837 (1971).
- ⁵ Murray, J. P. and Youniss, J., *Child Dev.*, **39**, 1259 (1968).
- ⁶ Smedslund, J., *Child Dev.*, **34**, 389 (1963).
- ⁷ Youniss, J., and Furth, H. G., *Child Dev.*, **36**, 534 (1965).
- ⁸ Furth, H. G., and Youniss, J., *Child Dev.*, **37**, 73 (1966).
- ⁹ Huttenlocher, J., *Psychol. Rev.*, **75**, 550 (1968).
- ¹⁰ Inhelder, B., Bovet, M., Sinclair, H., and Smock, C. D., *Am. Psychol.*, **21**, 160 (1966).
- ¹¹ Lehrman, D. S., in *Development and Evolution of Behavior* (edit. by Aronson, L. R., Tobach, E., Lehrman, D. S., and Rosenblatt, J. S.) (W. H. Freeman, San Francisco, 1970).

DR BRYANT replies: Youniss and Furth make three main criticisms of our article¹, all of which seem to me to be misguided. The criticisms are that we did not manipulate the memory variable, that the inferences which we detected may have been "sub-logical" and dependent on imagery and that Piaget's techniques did not involve memory and therefore are not open to the criticism which we make of them. I shall take these points in order.

We concluded that memory was the variable which led to failures in other experiments and not in ours, because no care was taken in these experiments to ensure that the children remembered the information which they had to combine inferentially, while considerable care was taken in ours. This seemed to us the chief difference between our technique and those of others. Of course, these were the first two experiments in a series and in subsequent work I did manipulate the memory variable. By a trial by trial analysis of what the child remembered and of his ability to reach the correct inference, I was able to make a direct relation between memory and inferences. I showed that as soon as children remembered the necessary information they could combine it inferentially. This work is reported in a recent publication².

In our 1971 article¹, we showed that children could combine information in a logical transitive inference, which means that they could work out that $A > C$ from the information that $A > B$ and $B > C$. Whether this was done through imagery or by some other means of representation is another question which does not affect the logical status of the inference. Indeed, it is rather ironical that Youniss and Furth quote Huttenlocher³ at this point because she has done a great deal to show that adults often use spatial imagery to solve logical problems and that in doing so they are being perfectly logical. There are no grounds for thinking, as Youniss and Furth suggest without any substantiation, that the use of spatial imagery in these problems is "sub-logical".

Their suggestion that Piaget's techniques did not involve memory is most surprising. Their "rereading" of Piaget's work has not been complete and they have missed a basic distinction made by Piaget himself between experiments of the type which we are criticizing and experiments involving "spontaneous" inferences.

Piaget has done experiments in which children were shown first that $A > B$ or $A = B$, then that $B > C$ or $B = C$ and finally were asked to make an inference about A and C . He has given clear descriptions of such experiments in which the quantities involved were weight⁴, volume⁵ and size^{6,7}. These are the experiments which we are criticizing, as they involve memory as well as inferences.

Youniss and Furth's suggestion that Piaget did not use this technique is, therefore, inaccurate. They wrongly state that his experiments are only of the "spontaneous" variety where the child not only has to make an inference, but also has to provide for himself the information on which the information must be based. We are not criticizing these "spontaneous" experiments and, indeed, I have suggested elsewhere² that the conclusions drawn from them are basically correct.

Finally, a comment on the statement that Piaget's theory "seems fair game to almost any type of reductive analysis based on elementary processes like memory, attention, learning etc." In our paper we were not trying to reduce the theory; we were simply trying to point out that the statement that young children cannot make transitive inferences was based on poorly designed experiments. Youniss and Furth have done nothing to show that the design of the traditional experiments on transitive inferences is any better than we suggest it is. They seem to be saying that the breadth of Piaget's theory excuses him from carrying out properly designed experiments. I cannot agree with this uncritical approach.

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Received January 18, 1973.

¹ Bryant, P. E., and Trabasso, T., *Nature*, **232**, 456 (1971).

² Bryant, P. E., in *Constraints on Learning* (edit. by Hinde, R., and Hinde J.) (Academic, 1973).

³ Huttenlocher, J., *Psychol. Rev.*, **75**, 550 (1968).

⁴ Piaget, J., and Inhelder, B., *La development des quantites physiques chez l'enfant*, Ch. 10 and 11 (Delachaux and Niestle, Paris, 1941).

⁵ Piaget, J., *The Child's Conception of Time*, Ch. 5 (Routledge and Kegan Paul, London, 1969).

⁶ Piaget, J., *Mechanisms of Perception*, Ch. 7 (Routledge and Kegan Paul, London, 1969).

⁷ Piaget, J., *Genetic Epistemology*, Ch. 3 (Columbia Univ. Press, New York, 1970).

GENERAL

Gamma Distribution in Consumer Purchasing

A LONGSTANDING assumption in the theory of repeat buying has now been explained by results on consumers' brand switching behaviour first published a few years ago¹ together with more recent findings concerning the independence of brand choice and weight of purchasing.

The NBD theory of repeat buying for frequently bought branded consumer goods rests on two assumptions. (i) Purchases of a particular brand by a given consumer in successive equal time-periods are independent and follow a Poisson distribution with constant mean. (ii) The mean rate of purchasing varies from consumer to consumer and follows a gamma distribution in the whole population. One consequence of this model is that the frequency distribution of purchases of a brand for the whole population in any given time period should follow the negative binomial distribution (hence the

name of the NBD model). This and other deductions have generally been found to hold in thousands of cases over the past 15 years²⁻⁴.

There has been considerable evidence supporting the Poisson assumption, and showing that the NBD model is not sensitive to such departures from the Poisson assumption as do occur^{2,5}. There is also overwhelming empirical evidence that different consumers have different mean purchasing rates, but there has been no direct evidence for the assumption that these differences should follow a gamma distribution.

However, a direct explanation for the appearance of the gamma distribution is provided by applying a theorem of Mosimann⁶ to the observed regularities in consumer brand-choice behaviour. This theorem, based on a powerful characterization of the gamma distribution due to Lukacs⁷, is as follows: "Let Y_i ($i=1, 2, \dots, g$) be g non-degenerate and positive random variables, and suppose that they are inde-

pendently distributed. Let $W = \sum_{j=1}^g Y_j$ and define the random

variables P_i ($i=1, 2, \dots, g-1$) by $P_i = Y_i/W$. Then each of the $(g-1)$ random variables, P_i , is distributed independently of W if and only if all Y 's have gamma distributions with the same scale parameter."

Applying this to consumer purchasing for a product-field with g brands, Y_i represents the average rate of buying brand i across different consumers, W represents a consumer's rate of buying the product-field as a whole, and P_i the proportion of a consumer's total purchases which is devoted to brand i .

Now the brand-switching results reported earlier¹ provide evidence that the purchasing rates Y_i are nearly independent, the correlations between them being generally very small. There also is now extensive empirical evidence that the P_i are approximately independent of W ; for instance, the share of purchases devoted to each brand is generally much the same amongst light, medium and heavy buyers of the product-field².

These empirical results together with Mosimann's theorem explain why the variation across consumers of the rate of buying a brand should approximate a gamma distribution. It also provides a link between the theory of repeat-buying of a single item and that of brand choice between different items. The development of a comprehensive model of buyer behaviour embracing single-brand and multi-brand aspects is proceeding.

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Received March 26, 1973.

¹ Ehrenberg, A. S. C., and Goodhardt, G. J., *Nature*, **220**, 304 (1968).

² Ehrenberg, A. S. C., *Repeat-Buying: Theory and Applications* (North Holland, Amsterdam, 1972).

³ Chatfield, C., Ehrenberg, A. S. C., and Goodhardt, G. J., *J. Roy. Statist. Soc., A*, **129**, 317 (1966).

⁴ Ehrenberg, A. S. C., *Appl. Stat.*, **8**, 26 (1959).

⁵ Chatfield, C., and Goodhardt, G. J., *J. Amer. Stat. Assoc.*, **68**, 344 (1973).

⁶ Mosimann, J. E., *Biometrika*, **49**, 65 (1962).

⁷ Lukacs, E., *Ann. Math. Statist.*, **26**, 319 (1955).

Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Farming in British India

Agrarian Conditions in Northern India. By Elizabeth Whitcombe. Volume 1: *The United Provinces Under British Rule, 1860-1900.* Pp. xxviii+330. (University of California: Berkeley, Los Angeles and London, 1972.) \$12.

AFTER the "Mutiny" uprising of 1857-1858 the Crown finally took over full responsibility for the government of British India from the East India Company and with almost missionary zeal embarked on an endeavour to bring the benefits of western civilization and scientific and technical innovation to the subcontinent. India was clearly a potential source of great wealth for Britain but official policy would be to act on the model of the enlightened British landlord, conscious of his duties as well as his privileges. Through public works, especially irrigation, the productivity of the land was to be increased many-fold, thus bringing security, greater prosperity and increasing revenue to the administration.

Dr Whitcombe, in the first of her two-volume study of agrarian conditions in Northern India, describes the practical effects of this policy on the rural population of the North Western Provinces and Oudh (which together now comprise the state of Uttar Pradesh) in the 40 years after the "mutiny". That a book of this kind is at all possible is due to the existence of a mass of statistics and first-hand observation contained in numerous contemporary reports, most of which are now housed in the India Office Library and Records in London. Dr Whitcombe has studied these with great thoroughness. Many of the settlement officers and other authors of technical reports were perceptive and sensitive observers and were candid in their criticism of government policy. The author frequently quotes them verbatim and to good effect in documenting her account.

The book begins with a description of the existing pattern of rural life, the complex cropping sequences and intricate community structure, both of which had evolved to cope with the vagaries of the monsoon climate. The author then goes on to describe the development and effects of the massive irrigation programme which was based on the construction of the Ganges, East Jumna and Agra canals. The second half of the book is devoted to the revised tenancy laws which the administration introduced, the new "scientific"

evaluation of land for the purposes of revenue collection and the effects of both of these on the pattern of rural indebtedness. The final chapters describe the pressures and dilemmas which increasingly faced the courts and the whole administrative structure. At times the wealth of detail in the book tends to overwhelm the reader. The constant recourse to vernacular names in describing the elements of the agricultural system and the positions and functions within the social hierarchy demands frequent reference to the glossary. But in the end this attention to detail wins through and reinforces the authority of Dr Whitcombe's case.

The book presents a formidable indictment of British policy. The canals, it is true, had greatly increased agricultural production and had brought a new wealth, but at considerable cost. The autumn crops of millets and pulses, on which the majority of the population depended for its staple diet, were not adapted to artificial irrigation. The new production was in sugar-cane, cotton, indigo, opium and wheat destined for the cities and the export trade. The pressures to grow these more "valuable" crops frequently resulted in over-cropping of the good land while the poorer land was neglected and the traditional systems of well irrigation abandoned. Over vast areas high water tables and excessive deposits of salts on the surface of the soil were brought about by bad irrigation practices and poor drainage, the latter often caused by the alignment of the newly constructed roads and railways. When the droughts came the inferior soils could no longer be pressed into service to make up the deficiency in food grains and the mass of peasantry starved.

On top of all this the new standard rent rates and the charges for irrigation water were imposed with a rigidity which took no account of the annual variations in harvest, in marked contrast to the flexibility shown by previous arrangements. The problem of indebtedness became infinitely worse. The government, despite its activities in the pursuance of law and order and its massive public works programme, stopped short on principle at interfering with social structure and the free flow of trade. The *zamindars*, landlords under the new tenancy laws and responsible for collecting the government's 50% of the rent rate, soon found themselves insolvent and subject to compulsory sale of their land. In the end,

to avoid total collapse, the administration had to institute a system of debt release which allowed them to be reinstated. Public works construction, rent rate assessments, and the operation of the legal machinery necessitated a growing bureaucracy which swallowed up almost all of the revenue, creating a vicious circle from which little progress emerged.

The significance of this book is more than as an authoritative documentation of the agrarian conditions in one area during a brief period of history. Today India is experiencing the first benefits of the so-called "green and blue revolutions". If these are to have a greater success than was achieved by the British administration of a hundred years ago, then more account will have to be taken of the ecological and sociological constraints to technological change. In volume 2 of her work, Dr Whitcombe intends to extend her survey to the period from 1900 to independence, thus providing a full perspective against which to judge current plans and achievements. GORDON CONWAY

Cradle to Grave

Developmental Physiology and Aging. By P. S. Timiras. Pp. x+692. (Macmillan: New York; Collier-Macmillan: London, February 1973.) £9.95.

ENTHUSIASM over a textbook is sometimes justified, and this would seem to be the case here. Professor Timiras and her co-authors have produced a comprehensive study book on human development which would be welcome even if it were not unique in being the first to include a full discussion of ageing. The ageing section alone is the most comprehensive and up-to-date gerontological text now available, so the enterprise would seem to be beyond praise. Past attempts to summarize gerontology have been desultory, limited to fundamental biology or to pathology, and poorly integrated with the preceding study of maturation. This one covers all physical aspects of the ageing process, demography included, and even has a chapter on the pharmacological attack on ageing. The scope of this part of the work alone is remarkable. It is of a standard and accuracy to appeal both to students, who are increasingly in need of gerontology teaching, and to experts.

The quality of the developmental sections is similar; while the last half of

the book is necessary information for the geriatrician, the first half is an orientation in paediatrics, human genetics and basic medical biology, excellently judged for medical and non-medical use. The title is misleading in that developmental physiology is dealt with in detail in its own right, and not as a comment on age processes. If it has a deficiency, in the light of the rate at which biology is widening, it is the self-imposed one of avoiding psychosocial biology; the section on sex determination, for example, contains no reference to learning and sex roles, and the opportunity of relating human rearing and psychosexual development to primatology and the peculiarities of the human growth curve has not been taken, but this is presumably a matter of policy. Illustrations and tables are excellent, the index adequate, and the bibliographies mostly updated to 1970 or later. The existence of this book should do a great deal both for gerontology and for the teaching of medical biology in general—it reflects the quality of the excellent grounding in these fields which Professor Timiras's course at Berkeley has been giving, and makes it generally available to teachers and students. Its appearance here is most welcome.

A. COMFORT

Introducing Genetics

Genetics: a Basic Guide. By I. J. Pedder and E. G. Wynne. Pp. 183. (Hutchinson Educational: London, December 1972.) £1.90.

AN introductory text for schools and colleges, such as this book claims to be, should aim to satisfy several basic requirements. It should be stimulating, accurate, informative and easy to read. Unfortunately this particular book fails to make the grade on several counts. I found the style frequently pedantic and occasionally patronizing.

The book has ten chapters which follow the fashionable sequence: DNA, gene action, Mendel, linkage, sources of variation, mating systems, population genetics and applied genetics with a final chapter devoted to experimental methodology.

The treatment of gene action is competent and informative; there is enough detail to stimulate but not too much to assimilate. I think, however, that the problem of differential gene action in development warrants more than a single page of text, most of which is devoted to the *lac* operon. The chapters on transmission genetics are well set out but would have been better with the inclusion of some examples for readers to work out for themselves. It is a pity that the chapter on variation deals almost exclusively with

chromosomal aberrations, allowing only one page for a very uninspired section on gene mutation. Genes in populations is another topic which is covered well with plenty of relevant examples. I am not sure that I like human genetics to be included under the umbrella of applied genetics; surely in a book at this level it merits a separate chapter. The sections on plant and animal breeding are rather weak and will not convince many readers that genetics has practical relevance. Why not mention the "green revolution"?

The book contains several unfortunate errors and omissions. Twenty years after Watson and Crick it is inexcusable to diagram the double helix with chains of the same polarity and to indicate that it replicates by one half-helix acting as the template for a complementary strand, which is formed, apparently non-enzymatically, from a mixture of free bases and sugar phosphates. In my opinion it shows extreme nomenclatural conservatism to refer to linkage and recombination as "non-Mendelian inheritance". The photographs of mitosis and meiosis are good but the reader should not be left to guess the identity of the organisms used to make the preparations. Two other photographs are so poor as to be quite useless. One, of maize pollen grains segregating for waxy/non-waxy, has been reproduced to give all the intermediate shades from black to pale-grey. The other, which illustrates the segregation of black/white ascospores in *Sordaria fimicola*, does little justice to what is, or should be, an excellent demonstration of the segregation of the alleles of a single gene.

There are cheaper books which cover less ground but do it better, and others, not much more expensive, which are more comprehensive and readable. It may be significant that whereas the authors acknowledge sources of material and information there is no specific mention of anyone having edited or at least commented on the whole manuscript. I wonder if anyone did.

R. A. WOODS

Structured Programming

Structured Programming. By O. J. Dahl, E. W. Dijkstra, and C. A. R. Hoare. Pp. viii+220. (Academic: New York and London, October 1972.) £4.20; \$12.50.

CRITICISM, to me, carries a hint of superiority, and even an appreciation implies equality. I make no such claims and I indulge in this impertinence only because so very few are qualified to review the work of Professor Dijkstra, whom the publishers correctly call "one of the leading programmers of the day".

Structural Programming comprises three competent useful articles about very large computer programs. The first, a lucid presentation by Professor Dijkstra that is comprehensible given only a nodding acquaintance with programming, discusses some difficulties, particularly verification and proof, indicates mental aids which help in overcoming these, and suggests how a major program may be constructed. Interestingly, this involves a restricted topology. His process is highly structured, in many hierarchical levels of decreasing abstraction and increasing particularity, with minimal internal decision-making at each stage.

This facilitates comprehension, proof and modification, but it seems at some cost in the complexity of the construction—though there is no simple way to design large programs. He mentions the storage requirement/computation speed trade-off and illustrates grouping and sequencing in an elegant closing example. Large-scale programming is still more art than science. This note made it less so.

The second section goes into some detail on the structure and storage of data, and on the assumptions and choices that underlie these. It was illuminating and informative, yet I felt that it was wholly relevant only to a fairly high-grade specialist programmer.

Finally, a short monograph deals largely with certain features of the powerful high-level language SIMULA 67—a fact which should be mentioned on the dust-jacket and in the abstract—and illustrates the implementation of some of the concepts introduced in the earlier sections.

This book was irritating because it was too good to spoil ineptly. These articles do have links but they are primarily useful to quite different groups and they should never have been published together. The Dijkstra section will extend the understanding of almost anyone beyond the basic programming level; the rest is just not as general; and £4.20 is a lot for a single relevant part, particularly to the students and junior staff who most need it.

NOEL FALCONER

Addendum

THE following bibliography was omitted from the book review "Space Science" by R. L. F. Boyd (*Nature*, 243, 483; 1973):

Space Research: Physical and Technical Principles. By M. G. Krishkin. Translated from Russian. Pp. 314. (Jerusalem: Israel Program for Scientific Translations, 1972.) (Distributed by John Wiley and Sons, Ltd, Chichester.) £9.65.

Obituary

Dr E. Bretscher, CBE

DR EGON BRETSCHER, who died suddenly in Zurich on April 16, had worked continuously on the British atomic energy project for longer than almost anyone else when he retired in 1966 as head of the Nuclear Physics Division at the Atomic Energy Research Establishment, Harwell. His work before and after 1940 (when he became attached to the project) was especially wide ranging, covering both chemistry and physics, and pure and applied science.

Born in 1901, the son of a Zurich banker, Bretscher's higher education was at the Eidgenössisch Technische Hochschule (ETH) in chemical engineering. His continuing interest was organic chemistry and he went to Edinburgh to take his PhD. Back at the ETH, Debye arranged an assistantship for him in the Physics Department, where he was one of the first people to use dipole moments to understand the chemical structure of an organic molecule. In this period he met many of the famous physicists who came to work with Pauli. He was a keen mountaineer and in 1930 he was rock climbing with Felix Bloch, who slipped down a vertical face; Bretscher saved Bloch's life—an exploit which he referred to later as his greatest contribution to physics.

He was awarded, on the recommendation of Debye and Pauli, a Rockefeller Fellowship at the Cavendish Laboratory in Cambridge for the year 1934–35. Here he was introduced to Rutherford and nuclear physics. He returned to Cambridge in 1936 where he stayed until January 1944, first as a Clerk Maxwell Scholar and then as a lecturer in experimental physics. His physics was self-taught and his additional deep knowledge of chemistry, including radiochemistry, made him an experimentalist of "outstanding skill" (in A. P. French's words) and an especially valuable member of the Cavendish. In the prewar period he concentrated on the supposed "transuranic" elements produced by bombarding uranium or thorium with neutrons, and (with N. Feather) was one of the first in 1939 to identify the activity in question not as a "transuranic" element but as an unusual isotope of a common element, in this case $^{212}_{54}\text{Po}$ iodine. About the same time, with L. G. Cook, he demonstrated the recoil of

fission fragments by collecting them on various surfaces.

When the British Maud Committee began work in April 1940 to study the possibility of an atomic bomb, Bretscher was one of the physicists enlisted to help. He made two notable contributions. First, he wrote in December 1940 (with Feather) a report predicting that the new element 94, formed in the course of slow neutron reactions in uranium 238, would have a large fission cross section which would permit both the production of a super explosive mass and the use of uranium 238 for fission. Bretscher worked out a method of isolating element 93 if sufficient irradiation of the uranium could be achieved and he felt he could do the same for 94 when minute amounts were available. The Cambridge equipment, however, was too primitive for the necessary irradiation. Bretscher's second contribution was to devise an ingenious technique for measuring the cross section of uranium for fast neutrons (*Nature*, **213**, 961; 1967). In a different direction, he helped the late Dr D. E. Lea in the latter's study of the effects of radiation on biological specimens. From work on the lethal action of radiation on the bacterium *Escherichia coli*, they concluded that bacteria, like higher organisms, have heritable genes and estimated the number of these genes to be somewhat greater than 1,000.

Bretscher was one of the British atomic scientists sent early in 1944 to Los Alamos where he worked in Fermi's Advanced Development Division. There, he was responsible for the "monstrously large" measurements of the deuterium tritium reactions which foreshadowed the hydrogen bomb.

Bretscher returned to Britain and the newly created Harwell late in 1946 and thereafter became primarily a scientific administrator—first in temporary charge of the Chemistry Division and then, from 1948, as head of Nuclear Physics. Although he had a profound respect for pure science, he urged that Harwell's chief task was to serve the practical needs of atomic energy by providing the engineers with scientific information for building the first production plants and then nuclear reactors. An example of his practical gifts was the planning with H. Seligman of the experiment called "Seanuts", which established the conditions governing dilution and dispersion of effluents discharged into the sea off the Cumberland coast, an operation

which yielded valuable new scientific, as well as technical, information.

Convinced that work of the Nuclear Physics Division should be directed to the needs of reactor design, Bretscher held the view—then heretical but now generally accepted—that reactors should be designed from first principles, given an adequate knowledge of the requisite nuclear constants. His objectives were thus to systematize the requirements for nuclear data for reactors, to provide facilities for their measurement and to compile and evaluate the results of such measurements so that they were available in useful form. In 1950 he set up and chaired the Nuclear Reactor Data Committee consisting of nuclear and reactor physicists and chemists; it produced exceptionally close collaboration between "users" and "measurers". In 1955, the Tripartite Nuclear Cross Section Committee was formed to foster collaboration between the UK, the USA and Canada. Much of its success was due to Bretscher, who later chaired it. He greatly influenced the drafting of its terms of reference, and his enthusiasm and foresight, coupled with authority and integrity, produced cooperation on a very large scale. He was, *inter alia*, the first to recognize the practical importance of quantum effects in the slowing down of neutrons in reactor moderators and organized through the Committee a major collaborative effort on this. By 1963 the capability of other Western European countries was such that the organization was expanded into the European American Nuclear Data Committee which Bretscher chaired. Before his retirement he was on the organizing committee of the world-wide International Nuclear Data Committee.

Meanwhile, under Bretscher, the Nuclear Physics Division built up its equipment into one of the most powerful ensembles for applied nuclear physics in the world. Most notable among the machines used were the successively more powerful linear electron accelerators used as pulsed neutron sources, culminating in the — still unique — multiplying assembly known as the neutron booster. The Nuclear Physics Division was, moreover, the birthplace of several activities which reached their full development elsewhere—for example, the work on neutron scattering in solids and liquids and on ion implantation.

Bretscher's anxiety to maintain the highest scientific standards was seen

lower down the professional scale. Long before the Training Boards, he appreciated the importance of competent supporting staff for the scientists, and set up a Scientific Assistants Training School for youngsters straight from school and took a stimulating, personal interest in it.

The variety and practicality of Bretscher's work, and the fact that some of the most important was done in wartime, meant that he did not always receive the scientific appreciation due to him. He did not clamour for credit and never appended his name to papers published from Harwell.

In 1931 he married Hanni Greminger, a very gifted mathematical student at the ETH, and they had five children; all three sons do research in the biological sciences. His happiness with, and pride in, his family were the mainstays of his life.

Announcements

University News

Professor A. Williams has been appointed to the Lapworth Chair of Geology and Headship of the Department of Geology at the **University of Birmingham**.

Mr R. K. Elliott, has been appointed to a Chair in the School of Education and to the Headship of the Division of History and Philosophy of Education at the **University of Birmingham**.

Miscellaneous

Dr John C. Bowman has been appointed Director of the **Agricultural Research Council's Animal Breeding Research Organization**.

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

Common Insect Pests of Stored Food Products: a Guide to their Identification. By Dr. H. E. Hinton and Dr. A. Steven Corbett. Fifth edition. Pp. 62. (Economic Series, No. 15.) (London: British Museum (Natural History), 1972.) 50p. [262]

Civic Trust Awards 1972: County Borough and Large Burgh Arcas. Pp. 100. (London: Civic Trust, 17 Carlton House Terrace, SW1Y 5AW, 1973.) 75p. [262]

The Natural Environment Research Council. Publications Series B, No. 4: Research in the Atmospheric Sciences. Pp. 23. **Publications Series B, No. 5: Marine Wildlife Conservation: An Assessment of Evidence of a Threat to Marine Wildlife and the Need for Conservation Measures.** Pp. 39. **Publications Series C, No. 9: The Severn Estuary and the Bristol Channel—An Assessment of Present Knowledge.** Pp. 19. (London: The Natural Environment Research Council, 1972 and 1973.) [262]

University of Cambridge: Department of Applied Biology. Memoir No. 44: A summary of the papers published and research in progress by the members of the Department and its Associated Research Organisations during the period October 1st, 1971–September 30th 1972. Review Series No. 27: Biology as a Humanity. Pp. 24. (Cambridge: The University, 1972.) 15p. [13]

Forestry Commission. Trees and People. Pp. 10. **Booklet No. 36: Timber Measurement for Standing Sales Using Tariff Tables.** By G. J. Hamilton. Pp. 31. 14p. **Booklet No. 38: Common Trees.** Pp. 16. 8p net. **Bulletin 46: Forest of Dean Day Visitor Survey: An Analysis of the Demand for Day Visitor Facilities.** By Robert J. Colenutt and Roger M. Sidaway. Pp. vi+52+8 plates. 60p net. **Forest Record 85: The Coal Tit.** By A. J. Deadman. Pp. 14. 14p net. (London: HMSO, 1973.) [13]

The Association of Commonwealth Universities. Annual Report of the Council, 1971–72. Pp. 51. (London: Association of Commonwealth Universities, 1973.) [23]

London School of Hygiene and Tropical Medicine (University of London), incorporating the Ross Institute and the TUC Centenary Institute. Annual Report 1971/1972. Pp. 34. (London: TUC Centenary Institute of Occupational Health, 1973.) [23]

Computer Board for Universities and Research Councils: Report of the Computer Board for the period 1st April, 1970–31st March, 1972. (Cmd. 5220.) Pp. 21. (London: HMSO, 1973.) 16p net. [23]

University of Strathclyde. Annual Report, 1971/1972. Pp. 84. (Glasgow: University of Strathclyde, 1973.) [23]

Predatory Birds in Britain. By the Avian Predators Working Party. Pp. 64. (London: The British Field Sports Society; and The Council for Nature, 1973.) 36p. [23]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 264, No. 865: The Secretion and Structural Evolution of the Shell of Thecaeid Brachiopods. By A. Williams. Pp. 439–478+plates 40 to 53. £2.10; \$5.90. Vol. 264, No. 866: An Ultrastructural Study of Axonal Changes Following Constriction of Postganglionic Branches of the Superior Cervical Ganglion in the Rat. By Margaret R. Matthews. Pp. 479–508+plates 54–62. £1.40; \$3.90. (London: Royal Society of London, 1973.) [23]

The Zoological Record, 1968, Vol. 105, Section 13: Insecta. Compiled by the Staff of the Zoological Society of London. Pp. xi+747. (London: The Zoological Society of London, 1972.) [23]

Loughdon College of Further Education. Prospectus 1973/1974. Pp. 74. (Loughdon, Essex: Loughdon College of Further Education, 1973.) [53]

The University of Leeds. Publications and Titles of Theses, 1970/1971. Pp. 145. (Leeds: The University, 1972.) 65p. [63]

The Carnegie United Kingdom Trust. Fifty-ninth Annual Report, 1972. Pp. 24+13 photographs. (Dunfermline, Fife: The Carnegie United Kingdom Trust, Comely Park House, 1973.) [63]

Other Countries

Publications of l'Institut National pour l'Étude Agronomique du Congo. Caractérisation Morphologique et Physico-Chimique de Profils Typiques de l'Afrique Centrale. Par C. Sys. (Hors Serie.) Pp. 497. (Brussels: Institut National pour l'Étude Agronomique du Congo, 1972.) 1,000 francs. [262]

Smithsonian Contributions to Zoology. No. 132: Morphology, Behavior, and Histogenesis of the Pelagospira Larva of Phascolosoma agassizii (Sipuncula). By Mary E. Rice. Pp. iii+51 (14 plates). \$1.25. No. 135: **Freshwater Triclad (Turbellaria) of North America, V: The Genus Polycelis.** By Roman Kenk. Pp. iii+15. 45 cents. No. 138: **Freshwater Triclad (Turbellaria) of North America, VI: The Genus Dendrocoelopsis.** By Roman Kenk. Pp. iii+16. 45 cents. **Smithsonian Contributions to Paleobiology, No. 14: Oermlian Branchiopods of West Texas, I.** By G. Arthur Cooper and Richard E. Grant. Pp. 230 (23 plates). \$2.25. (Washington, DC: Smithsonian Institution Press, 1972 and 1973.) [262]

Girard Estate Coal Lands in Pennsylvania, 1801–1884. By John N. Hoffman. (Smithsonian Studies in History and Technology, No. 15.) Pp. 86. (Washington, DC: Smithsonian Institution Press, 1972.) [262]

Australian Journal of Botany, Supplementary Series No. 6: The Palynology of Some Tertiary Pleistocene Deposits, Lachlan River Valley, New South Wales. By Helene A. Martin. Pp. 57. (East Melbourne: Editorial and Publications Section, CSIRO, 372 Albert Street, 1973.) [13]

Australia: Department of Supply. Defence Standards Laboratories Annual Report, 1971/1972. Pp. 87. (Ascot Vale, Victoria: Chief Superintendent, Defence Standards Laboratories, 1972.) [13]

Rubber Research Institute of Malaya. Annual Report 1971. Pp. 168. (Kuala Lumpur: Rubber Research Institute of Malaya, 1972.) \$3. [13]

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series, No. 31: Land Resources of the Vanimo Area, Papua, New Guinea. Comprising papers by E. Löffler, H. A. Haantjens, P. C. Heyligers, J. C. Saunders and Karen Short. Pp. 126+20 plates. Soil Publication No. 29: **Geology, Geomorphology, and Soils of Central County Hindmarsh (Mount Compass-Milang), South Australia.** By R. R. Maud. Pp. 85. (Melbourne: CSIRO, 1972.) [13]

Population Bulletin, Vol. 28, No. 4: Abortion—the Continuing Controversy. Pp. 1–32. (Washington, DC: Population Reference Bureau, Inc., 1972.) 50 cents. [13]

The International Nickel Company of Canada, Ltd. 1972 Annual Report. Pp. 32. (Sudbury, Ontario: The International Nickel Company of Canada, Ltd., 1973.) [13]

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